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HEMOGLOBIN, GLUCOSE, OXYGEN AND WATER IN THE ERYTHROCYTE

A Concept of Biological Magnitudes, Based upon Molecular Dimensions

By Dr. DAVID L. DRABKIN

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY, SCHOOL OF MEDICINE, UNIVERSITY OF PENNSYLVANIA

THE purpose of this communication is to direct the attention of biological workers to the illumination which may be afforded in many problems by expressing, when possible, biochemical relationships on a molecular basis.

The use of empirical "units" in the literature of the vitamins and hormones is justified in the early stages of investigation before precise quantities can be employed. However, in many instances the usage of "units" of this or that has continued long after the chemistry of the therapeutic or prophylactic agent has been established. The persistence of such empiricism is not only irksome, but serves to obstruct quantitative thinking and often delays proper interpretation and formation of useful concepts. With the enlarging interest in the architectural chemistry of cells and the dynamics of cellular metabolism, the realization appears inevitable that quantitation even on such a basis as "gm or mg per 100 ml" may have but limited usefulness. This is more obvious in a consideration of the complex equilibria which are the chemical mechanisms of cellular work and energy supply.

My attention was drawn forcibly to the advantages and desirability of calculating the concentration of cellular elements in terms of molecular populations

in the course of a study of the effect of fluoride and iodoacetate upon the disappearance of methemoglobin (oxidized hemoglobin), MHb, from blood on standing. In this *in vitro* system whole blood (with approximately 50 per cent. of the hemoglobin converted intracellularly to MHb) was employed in the presence of glucose, added to make a total concentration of about 300 mg per 100 ml. Upon incubation of such blood samples at 38° C in the presence of air, MHb reverted to oxyhemoglobin, and there was a parallel disappearance (glycolysis) of glucose. The addition of fluoride or iodoacetate inhibited both the glycolysis and the reversion of MHb. The inhibition owing to fluoride appeared to be partially overcome by the addition of pyruvate. In the interpretation of the significance of these findings the question arose as to the probability that a similar mechanism could function *in vivo*. The concentration of MHb in circulating blood is normally very low,[1] and when this pigment is produced in the organism in abnormal amounts it is removed rapidly from the circulation.[2] Is the interrelationship of glycolysis and MHb reduction physiological in view of the relative concentrations of glucose and hemoglobin in the erythrocyte? Our analytical values for adult human blood agree with the literature, and are for hemoglobin very high, approximately 34 gm per 100 ml (hematocrit packed volume) of red blood cells, and for glucose quite low, of the order of 0.075 gm per 100 ml of cells.[3] Expressed in these usual terms, the values apparently emphasize the overwhelming concentration of hemoglobin. But it will be seen that when calculations are made upon the basis of their respective molecular populations within the red blood cell, the concentrations of glucose and hemoglobin become virtually identical. It will also be apparent that the molecular population of even so "simple" a cell as the mammalian erythrocyte is closely packed. Hence, the interaction of these components in their cellular environment is rendered more probable.

Several examples of calculations follow. These were chosen in the fields most closely related to the writer's interests, and represent cases where definite data are available to make such calculations with some degree of confidence. No doubt many other examples can be found to supply equally instructive appraisals of metabolic phenomena within cells and tissues. It may be pointed out that the relationships to be discussed are implicit in values for concentration in molar terms, moles, M, per chosen volume.

[1] D. L. Drabkin and C. F. Schmidt, *Jour. Biol. Chem.*, 157: 69, 1945.

[2] W. W. Cox and W. B. Wendel, *Jour. Biol. Chem.*, 143: 331, 1942.

[3] M. Somogyi, *Jour. Biol. Chem.*, 78: 117, 1928.

1. *Calculation of Molecular Population of Hemoglobin in an Erythrocyte*

(*a*) Corpuscular volume (volume of individual cell in packed state): Erythrocyte count $= 5 \times 10^6$ per mm^3.

Hematocrit value = 45 per cent., or 45 ml per 100 ml of blood.

Packed cell count $= 5 \times 10^6$ per 0.43 mm^3 or per 4.5×10^8 μ^3 (since 1 $mm^3 = 10^9$ μ^3).

Therefore, volume of 1 cell $= 4.5 \times 10^8 / 5 \times 10^6 = 90$ μ^3 (90 cubic microns).

(*b*) Cellular hemoglobin concentration = 340 gm per 1,000 ml or per 10^{15} μ^3 (since 1 ml = 1 $cm^3 = 10^9$ $mm^3 = 10^{12}$ μ^3). Hence, per cell of 90 μ^3 volume the hemoglobin concentration is 30.6×10^{-12} gm, or 30.6 micro-micrograms ($340 \times 90/10^{15}$).

Since the molecular weight of hemoglobin is 66,700 (on the basis of 0.335 per cent. of Fe and 4 atoms of Fe), the molar concentration of the pigment = $340/66{,}700 = 5.1 \times 10^{-3}$ M per 10^{15} μ^3.

Therefore, since 66,700 gm of hemoglobin contain 6.02×10^{23} molecules (Avogadro's number—number of molecules per gram molecular weight), the molecular population in hemoglobin of a single red blood cell with a volume of 90 $\mu^3 = 5.1 \times 10^{-3} \times 6.02 \times 10^{23} \times 90/10^{15} = 2.76 \times 10^8$, or approximately 300,000,000 molecules of hemoglobin per cell. In this calculation the molecular weight is that of the non-hydrated protein. According to x-ray measurements,[4] wet hemoglobin has a molecular magnitude of approximately 132,000. If the latter value were employed the original concentration of hemoglobin in gm per liter of cells would have been taken as 673 gm (from $340 \times 132{,}000/66{,}700$). Hence, the same final result would be yielded in the calculation of molecular population. However, the hydration of the molecule may have to be considered in a calculation of the "space occupied" by the hemoglobin in the cell (see 3*c*).

2. *Calculation of Molecular Population of Glucose in a Red Blood Cell*

Cellular glucose concentration = 0.75 gm per 1,000 ml or per 10^{15} μ^3.

Hence, the glucose concentration per corpuscle of 90 μ^3 volume $= 0.75 \times 90/10^{15}$ gm $= 6.75 \times 10^{-14}$ gm.

Therefore, per cell the molar concentration of glucose $= 6.75 \times 10^{-14}/180 = 3.75 \times 10^{-16}$ M, and the corresponding molecular population $= 3.75 \times 10^{-16} \times 6.02 \times 10^{23} = 2.26 \times 10^8$, or approximately 250,000,000 molecules of glucose per cell. Thus, calculations 1 and 2 reveal that the molecular populations of hemoglobin and glucose within the erythrocyte are practically

[4] M. F. Perutz, Ph.D., Thesis, University of Cambridge, 1940; *Nature*, 143: 73, 1939; 149: 491; 150: 324, 1942.

identical. This relationship is not at all obvious in a comparison of the values for the concentrations of hemoglobin and glucose, when they are expressed in the customary manner as respectively 34 gm and 0.075 gm per 100 ml of cells.

3. *Calculation of the Number of Hemoglobin Molecules Which Can Crowd, Closely Packed, at the Surface of an Erythrocyte*

Our spectrophotometric measurements upon whole blood[5] by means of a special cuvette of 0.007 cm depth[6] are compatible with the view that hemoglobin in the cell may be present in solution. On the other hand, various speculations are current concerning the state of hemoglobin within the red blood corpuscle. Among them is the opinion, originally expressed by Bürker,[7] that hemoglobin "is held in the corpuscle by union with the membrane."[8] This deduction arose from the interpretation of the finding that the content of the blood pigment per cell varied in different mammalian species, but was relatively constant, 2.9 to 3.4×10^{-13} gm per μ^2 unit of surface of the erythrocyte.[7] Interesting as the relation of cellular hemoglobin and corpuscular area may be, it should be clear that the use of surface area in this connection is one of personal bias. The same constancy of hemoglobin content would have been obtained if cellular hemoglobin had been calculated on the basis of cellular volume. The hypothesis of "the remarkable distribution of the coloring matter of blood on the surface of the corpuscles"[7] has found acceptance in textbooks, and has invited further speculation concerning an appropriate shape for the hemoglobin molecule—to fit it for comfortable residence at the surface of the erythrocyte—such as "long prisms, attached at one end to the membrane of cholesterol-lecithin-protein."[8] A possibly less romantic but more satisfactory shape, a rather stubby ellipsoidal platelet or slab (unaffected by the degree of hydration of the crystal) with axes $x = y = 23$ A (Ångstroms) and $z = 37.6$ A, may be deduced for the hemoglobin molecule from x-ray crystallographic data now available upon horse MHb.[4] While it may be questioned whether hemoglobin molecules within the cell need have the dimensions deduced from the crystalline material, it would appear desirable to base present inferences upon measurement rather than upon intuition. The following calculations outlaw the theory that all or a considerable proportion of cellular hemoglobin is a constituent of the membrane. Optical evidence,[9] presented in support of a variant of the above hypothesis, namely, that hemoglobin is bound to "stromatin" (stroma protein), has been refuted.[10]

(a) Surface-volume relationship of red blood cell: Owing to the peculiar biconcave disc shape of the erythrocyte, its surface area is difficult to determine with exactness, but is of the order of 140 μ^2.

The diameter, D, of a wet human erythrocyte[11] = 8.5 μ. The area (by an approximation equation[11]) = $2.4\pi(D/2)^2 = 7.55(D/2)^2 = 7.55 \times 18.07 = 136.3$ μ^2, or 1.363×10^{-10} m^2 (since 1 $\mu^2 = 10^{-12}$ m^2). (This apparently small quantity adds up to a large value for the total surface of the erythrocytes in the body. A man of 70 kilos with a blood volume of 5.6 l. has 2.8×10^{13} erythrocytes (5×10^6 per mm^3 of blood). The total surface area of the red blood cells is, therefore, $1.363 \times 10^{-10} \times 2.8 \times 10^{13} = 3.82 \times 10^3$ m^2, or 3,820 square meters, a value 2,000 times greater than the surface area of the body, which may be taken as 1.87 m^2 for a man of 70 kilos and 178 cm height). It is obvious that a sphere of the same volume as the biconcave disc erythrocyte would have a smaller surface than the latter. In the presence of added lecithin the red blood cell assumes a spherical form without change in volume, which remains at 90 μ^3 (see 1 a). The surface area of this spheroidal cell may be calculated.

Volume, V, of spherical cell = $1.333\ \pi(D/2)^3 = 4.189(D/2)^3$.

Hence, $(D/2)^3 = V/4.189 = 90/4.189 = 21.47$ μ^3, and $(D/2) = 2.78$ μ.

Therefore, surface of spherical cell = $4\pi(D/2)^2 = 12.57(D/2)^2 = 12.57 \times 7.73 = 97.3$ μ^2, or surface = $3V/(D/2) = 3 \times 90/2.78 = 97.3$ μ^2. This area is smaller than that of the corresponding biconcave disc cell of the same volume by a factor of 1.4 (calculated from 136.3/97.3). It may be assumed that the red blood cell gets along with its peculiar shape, and various physical as well as physiological advantages have been attributed[11] to the biconcave disc form. Whatever the merits of this shape may be, it is clear that it provides a larger surface per volume. Thus, per hemoglobin molecule or per any other molecule within the erythrocyte a larger cell surface is available in the biconcave disc in comparison with the spheroid of the same volume. This fact, however, should not be assumed to favor the concept of membrane-bound hemoglobin, though it may have a bearing upon the diffusion of substances, such as oxygen, from the periphery into the interior of the cell.

[5] D. L. Drabkin and R. B. Singer, *Jour. Biol. Chem.*, 129: 739, 1939.

[6] D. L. Drabkin and J. H. Austin, *Jour. Biol. Chem.*, 112: 105, 1935–36.

[7] K. Bürker, *Sitzb. preuss. Akad. Wiss.*, 140, 1922; *Naturwissenschaften*, 11: 512, 1923.

[8] A. P. Mathews, "Physiological Chemistry. A Text-Book for Students," 6th edition, Baltimore, pp. 744–745.

[9] G. A. Adams, *Biochem. Jour.*, 32: 646, 1938.

[10] D. Keilin and E. F. Hartree, *Nature*, 148: 75, 1941.

[11] E. Ponder, in O. Glasser, "Medical Physics," Chicago, 1944, p. 1203.

(*b*) The number of molecules of hemoglobin which can be packed next to each other at the surface of a red blood cell:

Surface area of erythrocyte = 136.3 μ^2 = 1.363×10^{10} A^2 (since 1 μ^2 = 10^8 A^2).

The dimensions of the hemoglobin molecule, based on x-ray crystallographic measurements of the unit cell of horse MHb, containing 2 molecules (Perutz[4]), are: length (*b*-axis) = 64 A, width (in direction of *a*-axis) = approximately 48 A, and thickness (perpendicular to *c* plane) = 36 A. Assuming that the smallest surface of the molecule is oriented towards or attached to the corpuscle membrane, and treating it as a quadrilateral, an approximation of this area = $48 \times 36 = 1.728 \times 10^3$ A^2. Therefore, the largest number of hemoglobin molecules which conceivably could be accommodated at the surface of the erythrocyte = $1.363 \times 10^{10}/1.728 \times 10^3 = 7.89 \times 10^6$, or approximately 8,000,000 molecules. This is less than 3 per cent. of the total number of hemoglobin molecules in the red corpuscle ($7.89 \times 10^6 \times 100/2.76 \times 10^8 = 2.86$ per cent.), and the calculation no doubt overestimates the number of molecules of hemoglobin which can crowd together in a given space. The hemoglobin molecules would be packed tighter than sardines—with no room for oil. No account has been taken of such factors as the water of hydration of hemoglobin, or the influence of the presence of other molecular species within the erythrocyte which probably would operate against the possibility that a single species of molecules could collect side by side. It may be pointed out that although the hemoglobin molecule is a relatively rigid structure,[4] the red corpuscle is not. If the biconcave disc should assume a spheroidal form (a reversible process), there would be a contraction in surface to 9.73×10^9 A^2 (see above, 3 *a*). This must lead to a tighter packing of materials fixed at the surface—an impossibility if the rigid structures are already tightly packed side by side—or there must be a displacement of substances from the surface to the interior of the erythrocyte. It is therefore probable that only 2 per cent. or less of the total hemoglobin ($9.73 \times 10^9/1.728 \times 10^3 = 5.62 \times 10^6$, out of a total of 2.76×10^8 molecules) can be anchored at the surface of the red cell, and the non-rigidity of the corpuscular structure appears to be the best argument against membrane-bound hemoglobin.

(*c*) The volume occupied by corpuscular hemoglobin:

The percentage of cellular volume occupied by hemoglobin may be calculated from the grams of hemoglobin per 100 ml of corpuscles divided by the density of hemoglobin in solution[12] = 34/1.344 = 25.3 per cent. This value is consistent with that of 70.7 per cent. for corpuscular water (from 71 gm of water per 100 ml of erythrocytes[13] times the density of water at 38° C = 0.9954), and leaves a value of 4 per cent. for the space occupied by all the other constituents of the red blood cell. An appreciably tighter squeeze than this may be inferred from calculations based on the molecular volume of hemoglobin, deduced from the x-ray dimensions,[4] $= 64 \times 48 \times 36 = 1.11 \times 10^5$ A^3, which gives a value which is 34 per cent. of the volume of the corpuscle ($1.11 \times 10^5 \times 2.76 \times 10^8 \times 100/9 \times 10^{13} = 34$ per cent.). In calculating the above volume, the molecular shape was assumed to be that of a quadrilateral slab. Since the form of the hemoglobin molecule is rather that of an ellipsoid,[4] the volume is probably smaller, and, making an allowance of 15 per cent., $= 9.4 \times 10^4$ A^3. This yields a value = 28.8 per cent. of the corpuscular volume, and affords only 0.5 per cent. of the total space for all the other erythrocyte populations besides hemoglobin and water. Hence, with inferences based on deductions from x-ray data, whether the value of 34 or 29 per cent. is chosen for the percentage of total space needed for the accommodation of hemoglobin, it appears that some water molecules must occupy the same space as hemoglobin molecules, *i.e.*, that the hemoglobin molecule is "hydrated" within the corpuscle. The above x-ray data are upon air-dried hemoglobin, which probably contains some residual water. Its specific gravity is 1.27 rather than 1.344. The lower density has been explained[12] as mainly owing to the hydration of the crystal, and the best recent measurements suggest[14] that protein crystals may contain appreciable water of hydration, from 0.5 to 0.8 gm per gm of protein. Such values applied to hemoglobin would approximately double the molecular volume. However, the hydration of the crystal affords little insight into the state of hydration of the protein in solution. Only negligible information is available as to the intimacy of association of water and a protein such as hemoglobin in solution. If the water of hydration of a protein in solution be defined as "bound" in the sense that the molecules of water associated with the protein no longer exercise their normal solvent properties, it must be concluded from accurate vapor pressure measurements[15] on centrifuged corpuscles that such water of hydration is of negligible magnitude and that the total water of the erythrocyte is practically all "free." On the other hand, it is recognized[14] that

[12] G. S. Adair and M. E. Adair, *Proc. Roy. Soc. Lond.*, B 120: 422, 1936.

[13] P. M. Hald and A. J. Eisenman, *Jour. Biol. Chem.*, 118: 275, 1937.

[14] D. Crowfoot, *Chem. Rev.*, 28: 215, 1941; T. L. McMeekin and R. C. Warner, *Jour. Am. Chem. Soc.*, 64: 2393, 1942.

[15] A. V. Hill, *Proc. Roy. Soc. London.*, B 106: 477, 1930.

only water but salts can permeate the protein crystal structure, and it has been postulated[16] that if not water *per se* but water and solutes become associated with protein in solution, measurements involving the colligative properties would not disclose the true situation. It may well be that the ability of water plus solutes to permeate into spaces within the protein molecule may endow a special functional significance upon proteins in the cellular architecture. At any rate, there can be no doubt that even a simple cell such as the mammalian erythrocyte has a closely packed molecular population, affording intimate contact of molecules of different species. The question whether corpuscular hemoglobin is in solution or in some metastable state approaching incipient crystallization, as it appears to be in the erythrocyte of the white rat, can not be decided at present.

4. *Calculation of Molecular Populations of Dissolved and of "Bound" Oxygen, O_2, in an Erythrocyte*

(*a*) Dissolved O_2: From the absorption or solubility coefficient, α_{O_2}, at 38° C in plasma = 0.024 (*i.e.*, 0.024 ml of gas dissolved in 1 ml, at N.T.P.), and the partial pressure of O_2 in arterial blood = 97 mm of Hg, the amount of oxygen in solution in blood plasma is reckoned: $0.024 \times 100 \times 97/760 = 0.306$ ml per 100 ml of plasma, or 3.06 ml per 1,000 ml or per 10^{15} μ^3. Assuming equilibrium in respect to dissolved O_2 between the water of plasma and water of the corpuscles, the volume of corpuscular dissolved O_2 is taken as proportional to the respective percentages of water, 93.7 per cent. for plasma and 71 per cent. for cells.[13] Hence, oxygen in solution in the corpuscles $= 3.06 \times 71/93.7 = 2.32$ ml per 10^{15} μ^3, and dissolved O_2 per corpuscle of 90 μ^3 volume $= 2.32 \times 90/10^{15} = 2.09 \times 10^{-13}$ ml, at 38° C. (This value may be an overestimation. It implies that the water of the red cells is as free to dissolve O_2 as the water of plasma. Direct determinations of dissolved oxygen in erythrocytes, presumably owing to difficulties in such analyses, are unavailable. An inert gas, hydrogen, is 93 per cent. as soluble in corpuscles as in serum[17].) Since 1 gram mole of gas occupies 22,400 ml, at N.T.P., the molar concentration of dissolved $O_2 = 2.09 \times 10^{-13}/2.24 \times 10^4 = 9.33 \times 10^{-18}$ M per red blood cell.

Therefore, the molecular population in dissolved O_2 of a single erythrocyte $= 6.02 \times 10^{23} \times 9.33 \times 10^{-18} = 5.62 \times 10^6$, or approximately only 5,500,000 molecules of dissolved oxygen per arterial corpuscle.

(*b*) Molecules of O_2 "bound" to hemoglobin, and ratio of bound to free O_2 in arterial cells: The hemoglobin molecule contains 4 atoms of Fe, and, at full saturation (complete conversion to oxyhemoglobin), there will be 4 molecules of O_2 bound per 4 atoms of Fe in 1 molecule of hemoglobin. With the values of 98 per cent. for the saturation of arterial hemoglobin[1] and 2.76×10^8 for the number of hemoglobin molecules per cell (see 1 *b*), the bound $O_2 = 2.76 \times 10^8 \times 4 \times 0.98 = 1.08 \times 10^9$, or approximately 1,000,000,000 molecules of bound oxygen per corpuscle in arterial blood. Therefore, in an arterial erythrocyte the ratio of bound O_2 to free $O_2 = 1.08 \times 10^9/5.62 \times 10^6 = 192/1$. In other words, in a red corpuscle of arterial blood there is 192 times more oxygen united with hemoglobin than there is free in solution (physically dissolved). In venous blood, at 38° C, with 80 per cent. saturation of hemoglobin and an oxygen tension of 40 mm of Hg, one may calculate (in the same way as above) that there are, per corpuscle, 8.84×10^8 molecules of bound to only 2.31×10^6 molecules of free O_2, yielding a ratio of bound O_2 to free O_2 of 383/1. Hence, in a red cell of venous blood there is 383 times more oxygen combined with hemoglobin than there is free in solution. Such values are more pertinent and revealing with reference to the nature of the hemoglobin-oxygen equilibrium than values for bound and free oxygen in terms of volumes per cent. in whole blood, particularly when the value for free O_2 refers to plasma alone. Representative values in terms of volumes per cent. are: 20.5 for oxygen capacity (related to complete saturation of 15.3 gm of hemoglobin per 100 ml of whole blood) and 0.306 for free oxygen in 100 ml of plasma. The ratio of $20.5/0.306 = 67/1$ does not reveal, without the application of suitable corrections, the situation in respect to bound and free oxygen in the erythrocyte.

The number of molecules of dissolved O_2 in an erythrocyte, as disclosed by the calculations, is surprisingly small. Under normal conditions, in arterial blood corpuscles there is just enough oxygen to nearly saturate the hemoglobin, but not quite. With a minimum utilization rate of about 250 ml of O_2 per minute for an adult man at rest, and up to 2,500 ml per minute in strenuous exercise, and with at most only 15.4 ml of total dissolved O_2 in 5.6 liters of blood (70 kilo man, with 3.08 liters of plasma and 2.52 liters of cells), it is obvious that in providing oxygen a cooperative effort must exist among the carrier (hemoglobin), the pumping system (heart and circulation) and the source (respiration). So far as the carrier is concerned two factors are of dominant importance—(1) provision of sufficient dissolved oxygen in time, and (2) maintenance of an active form of hemoglobin, capable of uniting reversibly with oxygen. Physiologists do not appear to

[16] B. S. Neuhausen, *Jour. Biol. Chem.*, 51: 435, 1922.
[17] D. D. Van Slyke and J. Sendroy, Jr., *Jour. Biol. Chem.*, 78: 801, 1928.

have stressed the fact that functionally the erythrocyte is peculiarly adapted to aid hemoglobin in both of the above connections. The red blood corpuscle has a very low, almost negligible oxidative metabolism (consumes very little oxygen) in comparison with other cells. Indeed, it has been described, unfortunately, as "dead." If the erythrocyte had an active oxidative metabolism it would itself consume the dissolved oxygen (of which there is none to spare), and hemoglobin would be useless as a carrier. To illustrate what could occur if cells which consumed oxygen actively were present in blood, the following simple and instructive experiment may be performed: Mix equal parts of a solution (1 volume of cells in a total of 10 volumes), prepared from washed erythrocytes (100 per cent. of oxyhemoglobin), with a 10 per cent. suspension of washed, tapioca-free yeast. Dilute 1 volume of the mixture to a total of 2.5 volumes with distilled water, and allow to stand in an open test-tube at room temperature for 20 minutes. The color of the mixture changes to deep purple, owing to the formation of deoxygenated (reduced) hemoglobin. The deoxygenation of the hemoglobin with an active yeast suspension will be practically complete. (The yeast may be centrifuged off, and the hemoglobin-oxyhemoglobin mixture determined spectrophotometrically.) If the tube is shaken with air, the upper layer turns red (reoxygenation of the hemoglobin). On further standing reversion to reduced hemoglobin again occurs. This reversible change may be induced many times. To show that the phenomenon is that of oxygen consumption by the yeast cells rather than reduction by some active reducing agent in the yeast, a control experiment may be carried out, using a 1:10 solution of MHb, prepared by adding an equivalent of ferricyanide to the blood sample. The yeast now has no effect upon the color of the solution. MHb is not reduced by any substance in the yeast. If the yeast is separated from the MHb solution by centrifuging and added to a solution of oxyhemoglobin, the process of deoxygenation may again be demonstrated. Similar results can be obtained by substituting a fresh homogenate of liver for the yeast cells. It is, therefore, fortunate that mammalian red blood corpuscles do not have an active oxidative metabolism. Paradoxically, however, it is also fortunate that erythrocytes are not metabolically inert. They are provided with all the necessary biocatalysts—coenzymes I and II, the oxidizing enzyme, enolase, etc.—to support an active glycolytic metabolism. And, it is probable that glycolysis within the erythrocyte is an essential functional property, necessary for maintaining a high concentration of active hemoglobin *in vivo*. As mentioned in the opening paragraphs, glycolysis and disappearance of MHb (oxidized, inactive hemoglobin) go hand in hand. Iodoacetate and fluoride, known to poison certain of the above enzymes, inhibit both the breakdown of glucose and the reduction of MHb to active hemoglobin, capable of uniting with oxygen. In the conservation of dissolved oxygen the red corpuscle is merely a passive agent, in its capacity for shifting or keeping the equilibrium, oxyhemoglobin ⇌ hemoglobin ⇌ methemoglobin, far to the left; it has an active functional rôle, which deserves recognition in hemoglobin physiology.

5. *Calculation of Molecular Population of Water in an Erythrocyte*

There are 710 gm of water per 1,000 ml of blood corpuscles,[13] or per 10^{15} μ^3, and the mol. wt. of water = 18.02.

Hence, neglecting the density at 38° C = 0.993, the molar concentration of water = 710/18.02 = 39.4 per 10^{15} μ^3.

Therefore, the molecular population in water of a single corpuscle of 90 μ^3 volume = $39.4 \times 6.02 \times 10^{23}$ $90/10^{15} = 2.14 \times 10^{12}$, or approximately 2,000,000,000 molecules of water per red blood cell. This census indicates that water is by far the largest segment of the molecular population of cells. In the erythrocyte, which contains less water than many other types of cells, there is approximately an 8,000 times larger number of water molecules than molecules of hemoglobin ($2.14 \times 10^{12}/2.76 \times 10^8 = 7.75 \times 10^3$). An appreciably greater difference than this exists in the molecular populations of water and of dissolved oxygen, the ratio of the two components being 400,000 to 1, ($2.14 \times 10^{12}/5.62 \times 10^6 = 3.81 \times 10^5/1$). In other words, the number of molecules of free oxygen is less than 0.0003 per cent. of the number of molecules of water. Since water may not be an entirely silent partner in various metabolic transactions, relationships such as the above invite speculation.

6. *Relationship of Insulin and Glucose: the Relative Frequency of Two Simultaneous Events*

Thus far, the calculations have been limited to the molecular populations of the erythrocyte. Here, an example is furnished of the insight afforded into a more general biological relationship, that of insulin and glucose, when expressed in molecular terms. In a depancreatized dog a normal level of blood sugar can be maintained by a simultaneous, constant injection intravenously of *0.07 unit* of insulin and *0.25 gm* of glucose per kilo of body weight per hour,[18] the latter value representing the normal utilization rate

[18] S. Soskin and M. D. Allweiss, *Am. Jour. Physiol.*, 110: 4, 1934–35.

sugar. An approximately similar relationship of ulin and glucose is obtained from the hormone and gar requirements of a severely diabetic child. The lues of *0.07 unit* and *0.25 gm* are thus bracketed gether, and have some usefulness therapeutically, t they have little obvious further significance. owever, this empirically established relationship of ulin and glucose supplies fundamental data, needed the following calculations:

(*a*) Molar equivalent of 0.07 unit of insulin:

1 mg of pure, crystalline insulin = 22 units, and mol. . of insulin = 35,100.

Hence, 1 unit = 1/22 = 0.0454 mg, and 0.07 unit = 0454 × 0.07 = 0.00318 mg, or 3.18×10^{-6} gm.

Therefore, the molar equivalent of 0.07 unit of sulin = $3.18 \times 10^{-6}/3.51 \times 10^{4} = 9.06 \times 10^{-11}$ M.

(*b*) The molar equivalent of 0.25 gm of glucose nol. wt. = 180) = $0.25/180 = 1.39 \times 10^{-3}$ M.

Therefore, the molecular ratio of glucose to insu- = $1.39 \times 10^{-3}/9.06 \times 10^{-11} = 1.53 \times 10^{7}/1$. Thus, per olecule of insulin required by the completely dia- etic (or produced by the normal organism), 15,300,- 00 molecules of glucose are utilized (fully oxidized) r hour, or 255,000 molecules per minute. Attention may be drawn to the fact that care has been taken to state the relationship of insulin and glucose in such a way as not to imply that insulin is necessarily directly concerned with the oxidation of glucose. The exact functions and mode of action of insulin are still open questions. The calculations are of interest and valid as an indication of the relative frequency or magnitudes of two simultaneous processes, the production of insulin and the oxidation of glucose, for which some interrelationship is probable in the organism. If insulin is concerned with glucose oxidation, the value of 255,000 molecules of glucose taken care of by 1 molecule of insulin per minute assumes the character of a turnover number. As such, it is unusually high, and has the added distinction of being referable directly to the living organism. Insulin does a good job, the magnitude of which is defined clearly by the molecular relationship of the hormone and sugar.

Summary

Calculations have been presented to illustrate the insight which may be gained from the development of concepts of biological magnitudes upon the basis of molecular dimensions.

OBITUARY

RECENT DEATHS

ROBERT ELMER HORTON, consulting hydraulic engineer to the Tennessee Valley Authority and chairman of the Board of Consultants on Flood Control of the U. S. Department of Agriculture, died in his seventieth year on April 22.

DR. MARTIN H. ITTNER, chief chemist of the Colgate-Palmolive-Peet Company, died on April 22. He was seventy-four years old.

ARTHUR ROBERT HINKS, astronomer and since 1915 secretary of the Royal Geographical Society, died on April 18 at the age of seventy-one years.

DR. HANS SACHS, formerly professor of immunology at the Medical School of the University of Heidelberg, who was connected with Trinity College in Dublin, where he had a fellowship, died on March 28.

SIR AMBROSE FLEMING, known for his work in wireless, radio and telegraph developments, died on April 19 at the age of ninety-five years.

SCIENTIFIC EVENTS

NEW MECHANICAL ENGINEERING BUILDING AT THE CALIFORNIA INSTITUTE OF TECHNOLOGY

THERE has just been completed for the use of the Mechanical Engineering Department of the California Institute of Technology a five-story, reinforced concrete building. This follows the usual type of construction with three floors above the ground level and two floors below. However, the so-called first floor is five feet above the ground level so that by the use of light wells, daylight is supplied to the entire first basement, and because of a portion of the first basement floor being omitted, sunlight actually reaches the very lowest floor level. The building contains two very good drafting rooms on the top floor with excellent daylight lighting and also is illuminated by fluorescent lights. These lights are arranged on the ceiling in diagonals so that with the drafting tables located square with the room no shadows will be cast. There are two very good classrooms and a lecture room equipped with a projection lantern and screen and a demonstration table supplied with water, gas, compressed air and 110- and 220-volt AC current. The building also contains the offices for the members of the instructing staff and a good portion of the equipment of the Laboratory of Mechanical Engineer-

ing. The latter includes all the steam laboratory machinery, together with some other pieces, such as an air compressor, a refrigeration machine, a Servel gas-fired, air-conditioning unit for laboratory purposes, and similar items. Most of the internal combustion engineering equipment is left in the previous old laboratory, which is separated from any classrooms because it is noisier than the machinery just mentioned which is in the new building. Water that is used in the steam condenser and other cooling equipment is in turn cooled in a forced draft cooling tower on the room of the building.

R. L. DAUGHERTY

THE CORROSION RESEARCH LABORATORY OF THE ILLINOIS INSTITUTE OF TECHNOLOGY

THE war has been responsible for an increased interest in corrosion, due to the shortage of materials, the difficulty of replacement and the severe corrosion attack of semi-tropical climates.

The problem will become of even greater importance in the post-war period when many thousands of tons of intricately built equipment will be stored in government warehouses for immediate call on notice of from thirty days to perhaps twenty years. The corrosion protection measures involved will be on an unprecedented scale.

It was to meet the need for an educational and research center for studies on corrosion in this country that the Corrosion Research Laboratory was recently established at the Illinois Institute of Technology at Chicago. Dr. H. J. McDonald, associate professor of chemistry, has been appointed director of the new laboratory. At present the most extensive project under investigation is a study of the stress corrosion of mild steel.

The Illinois Institute of Technology provides space for the laboratory and its staff and a certain amount of funds for its equipment and operation. However, to continue the basic research program of the laboratory and to assure its permanency, industrial sponsorship of its fundamental research program will be sought.

THE ROCKEFELLER FOUNDATION[1]

ACCORDING to a review of the work in 1944 of the Rockefeller Foundation by Dr. Raymond B. Fosdick, president of the foundation, appropriations amounted to $10,306,258. This sum represents a substantial increase over the $7,760,186 appropriated in 1943. The income of the foundation from investments during the year was $8,209,807. This income was supplemented by a balance remaining from the preceding year.

[1] From review of work in 1944 of Dr. Raymond P. Fosdick of the Rockefeller Foundation.

The appropriations were distributed for the most part in five major fields, roughly as follows: Public health, $3,200,000; medical sciences, $1,253,000; natural sciences, $1,090,000; social sciences, $2,193,000; and humanities, $1,548,000.

Of the money appropriated during the year 7[illegible] per cent. was for work in the United States and 2[illegible] per cent. for work in other countries.

As fast as possible contacts are being reestablished in Europe. An officer of the foundation has been stationed in London throughout the war. In 1944 a staff member of the International Health Division was also assigned to the London office, and as soon as conditions permit, it is expected that he will establish headquarters in Paris. The director of the Division of Social Sciences spent two months in Great Britain, and in the latter part of the year representatives of the foundation were able to visit France. The Far Eastern office, formerly in Manila and now in Delhi, has been manned throughout the war. . . .

Ever since 1939 the Rockefeller Foundation has tried to keep in touch with as many as possible of the scholars and institutions of war-torn nations. Contact with countries like Norway, Denmark, Holland, Czechoslovakia and Poland has presented unsolvable difficulties, and only recently have relationships been reestablished in France. But over all this period, assistance has been continued to research projects and institutions in Great Britain, Sweden and Switzerland; and it is gratifying to report the extent to which fundamental work in science has been maintained. During 1944 continuing support was granted to eighteen projects in the natural sciences located in Europe. Of these, nine were in England, seven in Sweden and two in Switzerland. These projects were for the most part related to the application of the techniques of physics, chemistry and mathematics to biological problems.

No words can do justice to the devotion and gallantry with which much of this research has been carried on—sometimes in bombed-out laboratories and generally under conditions of hardship which would discourage all but the stoutest hearts. Shortages of materials and scientific literature, interruption of communication with other institutions, overcrowded laboratories due to the influx of refugee scientists—these are only a sample of the difficulties which have confronted the few remaining research centers in Europe.

But the experience of these years has proved once again that scientists everywhere speak the same great language of ideas—an international language of tolerance and hospitality for those who choose to hear. The letters which the foundation has received during the last few years bear eloquent testimony on this point. Professor Manne Siegbahn, of the Academy of Sciences in Stockholm, has opened the doors of his

laboratory to scientists driven out of Denmark, Finland and Norway. At the University of Stockholm, Professor John Runnström has crowded into his institute refugee scientists who have come from nine different nations. In Zurich, Professor L. Ruzicka, of the Technische Hochschule, has welcomed to his laboratory refugees who represent most of the occupied countries of Europe. In all these laboratories, reports from hosts and guests alike speak with deep satisfaction of the opportunity to continue their basic research in a world of confusion and catastrophe.

The work of Dr. Georg von Hevesy illustrates this same undaunted spirit. He has been twice a refugee. Driven out of the University of Freiburg in the early days of the Hitler regime, he went to the University of Copenhagen. Driven out of Copenhagen by the Nazis in 1943, he went to the University of Stockholm. In November, 1944, he was awarded the Nobel prize in chemistry.

THE AMERICAN PHILOSOPHICAL SOCIETY

THE following is the list of officers and members elected on April 19 by the American Philosophical Society, Philadelphia:

President: Thomas S. Gates.
Vice-presidents: William B. Dinsmoor, A. Newton Richards, Harlow Shapley.
Secretaries: W. F. G. Swann, Ernest M. Patterson.
Curator: John Story Jenks.
Treasurer: Fidelity-Philadelphia Trust Company.
Councillors (to serve for three years): Karl K. Darrow, *Class I;* Edwin G. Conklin, *Class II;* Nicholas Kelley, *Class III;* Carl W. Blegen, *Class IV.*

The following fifteen residents and five foreign residents were elected:

CLASS I—MATHEMATICAL AND PHYSICAL SCIENCES

Subrahmanyan Chandrasekhar, Williams Bay, Wisconsin.
Bradley Dewey, Cambridge, Massachusetts.
Carl Shipp Marvel, Urbana, Illinois.
J. Robert Oppenheimer, Berkeley, California.

Foreign

Harold Bohr, Copenhagen, Denmark.
Georges Lemaître, Louvain, Belgium.

CLASS II—GEOLOGICAL AND BIOLOGICAL SCIENCES

George Wells Beadle, Stanford University, California.
Edwin Garrigues Boring, Cambridge, Massachusetts.
Edwin Broun Fred, Madison, Wisconsin.
Elmer Verner McCollum, Baltimore, Maryland.

Foreign

Frederic Charles Bartlett, Cambridge, England.

CLASS III—SOCIAL SCIENCES

Herbert Heaton, Minneapolis, Minnesota.
Roy Franklin Nichols, Swarthmore, Pennsylvania.
Frank W(allace) Notestein, Princeton, New Jersey.
Donald Ramsey Young, Washington, D. C.

CLASS IV—HUMANITIES

Kemp Malone, Baltimore, Maryland.
Henry Ernest Sigerist, Baltimore, Maryland.
Lily Ross Taylor, Bryn Mawr, Pennsylvania.

Foreign

Walter Wilson Greg, Petworth, Sussex, England.
Alan J. B. Wace, Cambridge, England.

ELECTIONS OF THE NATIONAL ACADEMY OF SCIENCES

AT the annual meeting of the National Academy of Sciences, which was held in Washington on April 23 and 24, Dr. Luther P. Eisenhart was elected vice-president for a four-year term, ending June 30, 1949. Drs. W. M. Stanley and John T. Tate were elected members of the council for a three-year term, ending June 30, 1948.

The following members were elected:

Alfred Blalock, professor of surgery, the Johns Hopkins University.
Edward Hull Cochrane (Rear Admiral), chief of bureau of ships, in charge of design, construction, repair and maintenance of the Fleet, Washington, D. C.
Henry Eyring, professor of chemistry, Princeton University.
Enrico Fermi, professor of physics, Columbia University.
James L. Gamble, professor of pediatrics, Harvard Medical School.
Henry Gilman, professor of organic chemistry, Iowa State College.
Beno Gutenberg, professor of geophysics, California Institute of Technology.
Harold Hibbert, E. B. Eddy professor of industrial and cellulose chemistry, McGill University, Canada.
Mervin J. Kelly, executive vice-president, Bell Telephone Laboratories, New York City.
Victor K. La Mer, professor of inorganic chemistry, Columbia University.
George W. Lewis, director of aeronautical research, National Advisory Committee for Aeronautics, Washington, D. C.
Ralph Linton, professor and chairman of the department of anthropology, Columbia University.
Clarence Cook Little, director of the Roscoe B. Jackson Memorial Laboratory and managing director of the American Society for the Control of Cancer, Bar Harbor, Maine.
Paul C. Mangelsdorf, professor of economic botany and assistant director of the Botanical Museum, Harvard University.
John H. Mueller, professor of bacteriology and immunology, Harvard University.
John R. Paul, professor of preventive medicine, Yale University.
John B. Reeside, Jr., U. S. Geological Survey, in charge of paleontology and stratigraphy, Washington, D. C.

Francis W. Reichelderfer, chief, United States Weather Bureau.

William W. Rubey, chief, Division of Areal Geology and Basic Science, U. S. Geological Survey; chairman, Division of Geology and Geography, National Research Council.

Homer W. Smith, professor of physiology and director, Physiological Laboratory, New York University.

William C. Stadie, professor of research medicine, University of Pennsylvania.

Otto Stern, professor of physics and director of Research Laboratory of Molecular Physics, Carnegie Institute of Technology, Pittsburgh.

Harald Ulrick Sverdrup, director and professor of oceanography, Scripps Institution of Oceanography, La Jolla, Calif.

Cornelius B. van Niel, professor of microbiology, Hopkins Marine Station, Stanford University, Pacific Grove, Calif.

John C(harles) Walker, professor of plant pathology, University of Wisconsin.

Alexander Wetmore, secretary, Smithsonian Institution.

Hassler Whitney, associate professor of applied mathematics, Harvard University.

Eugene P. Wigner, Thomas D. Jones professor of physics, Princeton University.

Robert R. Williams, chemical director, Bell Telephone Laboratories, New York City.

Benjamin H. Willier, Henry Walters professor of zoology, School of Higher Studies, the Johns Hopkins University.

Foreign Associates

Sir (William) Lawrence Bragg, Cavendish professor of experimental physics, University of Cambridge.

Harold Jeffreys, F.R.S., fellow of St. John's College, Cambridge; university lecturer in mathematics.

Paul Karrer, professor of organic chemistry and director of the Chemical Institute, University of Zurich.

The Svedberg, professor of physical chemistry, University of Uppsala.

Sir Geoffrey I. Taylor, F.R.S., lecturer in mathematics and physics, Trinity College, Cambridge; Yarrow professor of the Royal Society in 1923.

SCIENTIFIC NOTES AND NEWS

THE Carty Gold Medal of the National Academy of Sciences, with an honorarium of $2,500, was presented at Washington on April 23 to Dr. W. F. Durand, professor of mechanical engineering emeritus of Stanford University, a member of the National Advisory Committee for Aeronautics. The medal is awarded every other year for "noteworthy and distinguished contributions in any field of science."

IN tribute to Dr. Frank R. Lillie, Andrew MacLeish distinguished service professor emeritus of embryology of the University of Chicago, the Frank R. Lillie room in the Hull Zoological Laboratory was dedicated on April 12, with Carl R. Moore, chairman of the department of zoology, officiating. Professor Lillie spoke briefly.

THE Bessemer Gold Medal of the British Iron and Steel Institute has been awarded to Harold Wright, chief metallurgist to Dorman, Long and Company, Ltd., Middlesbrough, in recognition of his "valuable contributions made over many years to improve the technique of iron and steel manufacture." The medal will be presented to him on May 9.

THE Association for the Study of Internal Secretions has announced the recipients of the Squibb and the Ciba awards made annually for outstanding researches in endocrinology. The award of E. R. Squibb and Sons was made to Dr. E. C. Kendall, of the Mayo Clinic and the Graduate School of the University of Minnesota, for his discovery of the active principle of the thyroid gland and the more recent fractionation of the hormones of the adrenal cortex. The award for younger investigators, made possible through the grant of Ciba Pharmaceutical Products, Inc., was given to Dr. Jane Anne Russell, of Yale University, for her work on carbohydrate metabolism as affected by the pituitary, the adrenal and the thyroid glands. Because of the cancellation of the 1945 annual meeting, the formal presentation of the awards will be made at a later date.

DR. CHARLES F. BROOKS, professor of meteorology and director of the Blue Hill Observatory of Harvard University, was recently honored at a dinner on the occasion of the conclusion of twenty-five years of service as secretary of the American Meteorological Society.

Nature reports that the Council of the Royal Society of Edinburgh has awarded the Gunning Victoria Jubilee Prize, for the period 1940–44, to Professor H. W. Turnbull, of the University of St. Andrews, for his distinguished contributions to mathematical science and the history of mathematics; and the Makdougall-Brisbane Prize, for the period 1942-44, jointly to Professor Max Born and Dr. H. W. Peng, of the University of Edinburgh, for their papers on "Quantum Mechanics of Fields" published in the *Proceedings* of the society within the period of the award.

SIR LAWRENCE BRAGG, Cavendish professor of experimental physics at the University of Cambridge, gave a lecture at the Fondation Universitaire in Brussels on science and technical work in relation to

civilization. On this occasion the medal of the foundation was presented to him.

THE latest issue of the *Record of the Academy of Sciences, U.S.S.R.*, is devoted to the achievements of the president of the Soviet Academy of Sciences, V. L. Komarov, on the occasion of his seventy-fifth birthday and the fiftieth anniversary of his scientific work. A number of articles describe his work as the leader and organizer of Soviet scientific activities and as the outstanding botanist of the U.S.S.R.

THE University of Cincinnati Chapter of Sigma Xi announces the following officers elected for the years 1945–47: *President,* Dr. George B. Barbour, dean of the McMicken College of Liberal Arts; *Vice-president,* W. C. Osterbrock, professor of electrical engineering; *Secretary-Treasurer,* Dr. Saul B. Arenson, professor of inorganic chemistry. Members of the nominating committee elected to serve for six years are Dr. H. J. Kesten, professor of biophysics, and Dr. H. L. Miller, professor of mathematics.

A CHAPTER of the Society of Sigma Xi was installed at the University of Connecticut at Storrs, on March 15. The installing officers were Dr. Harlow Shapley, national president of the society, and Dr. George A. Baitsell, executive secretary. Dr. Shapley gave the installation address and Dr. Baitsell addressed the group on the relation between the national and local societies. The following officers were elected: Joseph C. Shaw, *President;* H. M. Scott, *Vice-president;* Martha Potgieter, *Secretary-Treasurer.* Following the reception and dinner, a Sigma Xi lecture was given by Dr. Shapley, who spoke on "News from the Star Front."

DR. GEORGE HEPTING, pathologist at the Appalachian Forest Experiment Station, has been elected president of the Southern Shade Tree Conference.

DR. JOHN M. FOGG, JR., professor of botany and vice-provost of the University of Pennsylvania, has been elected to honorary membership in the Pi Gamma Mu National Social Science Honor Society. He gave an address entitled "Around the World in Thirty Minutes" at the annual meeting of the society on April 13.

DR. GEORGE S. CALLAWAY has been installed as president of the New York Academy of Medicine.

DR. GEORGE B. ROTH, since 1924 professor of pharmacology at the George Washington University School of Medicine, has retired with the title emeritus.

DR. HARRY WILLIAM MOUNTCASTLE will retire next June as head of the department of physics and astronomy of Western Reserve University, where he was appointed assistant professor of physics in 1907.

DR. ARTHUR HOLLY COMPTON, dean of the Division of Physical Sciences at the University of Chicago, has accepted the chancellorship of Washington University, St. Louis, and will assume the work as soon as he is freed from commitments to the Government involving scientific research connected with the war effort. Dr. Compton was head of the department of physics of Washington University from 1920 until 1923.

A DIVISION OF CANCER RESEARCH has been established at the College of Medicine of the Ohio State University. Dr. Charles A. Doan, dean of the medical college and director of medical research, has been appointed director, with Dr. Herman A. Hoster as associate director immediately in charge.

DR. O. N. ALLEN, formerly head of the department of bacteriology of the University of Hawaii, has become head of the department of bacteriology of the University of Maryland. He succeeds Dr. Lawrence H. James, who has opened a consulting laboratory in Chicago.

AT the School of Medicine of the University of Utah, Dr. Francis D. Gunn, formerly assistant professor of pathology at Northwestern University, has been appointed professor and head of the department of pathology. Dr. Louis S. Goodman, professor of pharmacology at the University of Vermont, has been appointed professor of pharmacology and chairman of the department of pharmacology and physiology. Dr. James E. P. Toman, instructor in physiology, also of the University of Vermont, has been appointed assistant professor of physiology.

NEW appointments in the department of animal husbandry of the Utah State Agricultural College include Dr. Louis L. Madsen, senior nutritionist, U. S. Bureau of Animal Industry, Beltsville, Md., as head of the department, and Dr. Carroll I. Draper, associate professor of poultry husbandry at the University of Hawaii, as associate professor of poultry husbandry.

DR. GUSTAVE J. NOBACK, professor of anatomy and chairman of the biological sciences in the Graduate School of New York University, has become dean of the Essex College of Medicine and Surgery of Newark.

DR. NELSON DALE, professor of geology at Hamilton College, Clinton, N. Y., has been appointed director of the college museum.

DR. K. A. C. ELLIOTT has joined the research staff of the Montreal Neurological Institute, with an appointment as assistant professor in neurochemistry at McGill University. He was formerly in charge of the Biochemical Research Laboratories of the Institute of the Pennsylvania Hospital, Philadelphia.

DR. D. S. MACLAGAN, lecturer in zoology at King's College, University of Durham, has been appointed head of the department of zoology of the West of Scotland College of Agriculture, Glasgow, and research advisory officer in agricultural zoology for the southwest of Scotland, in succession to Professor L. A. L. King, who has retired.

THE Commonwealth Fund has appropriated $8,200 to be used by Dr. Carl J. Wiggers and associates of the department of physiology of Western Reserve University for continuance of their studies on the peripheral circulation and shock during the coming year.

DR. GEORGE E. BURCH, associate professor of experimental medicine at the School of Medicine of Tulane University, New Orleans, has been appointed civilian consultant to the Surgeon General for diseases of peripheral blood vessels, with particular emphasis on trench foot and allied conditions.

DR. HAROLD H. SHEPARD, who since July, 1943, has been on leave of absence from the University Farm of the University of Minnesota to enable him to work with the Office of Materials and Facilities of the War Food Administration, in Washington, D. C., has become insect toxicologist of the New York State College of Agriculture, Cornell University. He succeeded Dr. Roy Hansberry, who joined the staff of the Shell Development Company, Modesto, Calif., in August, 1944. Dr. L. B. Norton, insecticide chemist formerly at the Geneva Experiment Station, is now stationed at Ithaca and will be associated with Dr. Shepard.

PROMOTIONS announced at the Central Laboratories of the General Foods Corporation include Dr. Willard Roberts, who will be director of the new section of food technology which takes the place of the sections of processing and cereal technology; Dr. Harold A. Campbell, assistant director of the engineering research section, who will have new divisional responsibilities for processing technology, chemical engineering, and processing engineering. The section of physical chemistry research, directed by L. W. Elder, will be enlarged to include the division of analytical chemistry directed by Dr. Martha Johnson. Dr. A. C. Shuman has been named assistant director of the section; he will aid Dr. Elder in the establishment of a new division of physical measurements, and he will also be in charge of microscopy and photography. Dr. T. R. Wood, research chemist, has been promoted to be head of the phytochemistry division of the section of organic chemistry.

DR. CORNELIUS P. RHOADS, director of the Memorial Hospital for the Treatment of Cancer and Allied Diseases, has returned after serving for nearly two years as chief of the Medical Division of the Chemical Warfare Service of the Army.

THE James Arthur Lecture on the evolution of the human brain, sponsored by the American Museum of Natural History, was delivered on April 30 in the lecture hall of the Roosevelt Memorial by Dr. K. S. Lashley, research professor of neuropsychology at Harvard University and director of the Yerkes Laboratories of Primate Biology. He spoke on "Neural Correlates of Intellect."

DR. CLARENCE S. ROSS, chief of the section of petrology of the U. S. Geological Survey, delivered on April 16 the Edward Orton, Jr., Fellowship Lecture before the dinner meeting of the upstate New York section and the forty-seventh annual meeting of the American Ceramic Society at Buffalo, N. Y.

DR. W. T. THOM, JR., professor and chairman of the department of geological engineering of Princeton University, recently addressed the fourth Industrial Planning Conference on Power Resources on "Energy Resources and National Power."

DR. JOHN F. MCINTOSH, secretary to the faculty of medicine of McGill University, spoke on April 4 to the members of the Osler Clinical Society at the Medical School of the University of Vermont. He discussed the problem of urinary calculi in ancient times and in a modern clinic.

DR. FRANCIS O. SCHMITT, professor of biology and biological engineering at the Massachusetts Institute of Technology, will deliver the eighth Harvey Society Lecture of the current series at the New York Academy of Medicine on May 17. He will speak on "Ultrastructure and the Problem of Cellular Organization."

THE alumni of the department of chemistry of the University of Kentucky has established a fund to be known as the "Ralph Nelson Maxson Memorial Fund," the purpose of which is to create an endowment for the library of the department of chemistry, supplementing the private library of Dr. Maxson, who was formerly head of the department, which was presented to the school following his death.

THE Abbott Laboratories, Chicago, have appropriated $50,000 to provide research fellowships in ten universities. These are the California Institute of Technology, Cornell University, Harvard University, the University of Illinois (Medical School), the Massachusetts Institute of Technology, the University of Minnesota, the Ohio State University, Purdue University, Tulane University of Louisiana and the University of Wisconsin.

IT is reported in *The Times*, London, that the Royal Society has been informed that the Treasury has pro-

ided in the estimates for the fiscal year 1945–46 for he following grants which are administered by the ociety: For scientific investigations, £14,000; for sci-ntific publication, £7,000; for scientific congresses, £1,600.

A CORRESPONDENT writes that "the Belgian Govern-ment Information Center reports that so far as they know the Congo Museum at Tervueren is in good con-dition. The Germans have spared this museum, and the same staff is still there."

THE daily press reports that to offset the continued isolation of the universities at Amsterdam, Delft, Groningen, Leyden and Utrecht—all of which are still in Nazi-occupied territory—the Netherlands Ministry of Education has arranged for temporary academic centers in the liberated area.

The Journal of Scientific and Industrial Research, Delhi, India, states that the Government of Bengal has decided to set up a Provincial Statistical Bureau. Each department of the government will be instructed to keep the bureau informed of its activities in statistical matters and to seek the guidance of the bureau when it proposes to introduce new schemes or methods. The function of the bureau will be primarily advisory in character. It will impart technical advice regarding the collection of primary statistics, classification, tabulation and analysis of statistical material and the form of publication of statistical data.

DISCUSSION

AMPLE EXERCISE AND A MINIMUM OF FOOD AS MEASURES FOR CANCER PREVENTION?

As measures for the prevention of human cancer a recent paper by Potter[1] advocated ample exercise, a minimum of food and "proper medical care so that any chronic irritations are eliminated." At the present time there is a paucity of information on the influence of dietary restriction upon the prevention of cancer in human beings. In the absence of data on man we must rely on the results of animal experiments for information on dietary means for delaying the onset of cancer, its partial or complete prevention, and then attempt to evaluate the possible effects of applying such procedures to the human population. Our present concept must, therefore, be largely influenced by this type of information without knowing to what extent it may be applicable to the prevention of cancer in another species, man.

Data from several sources are summarized in Table 1. This table sets forth the results of several dietary regimens that have been effective in decreasing the incidence of spontaneous mammary cancer in susceptible strains of mice and includes other effects which have been noted in the animals subjected to these regimens. Mammary tumorogenesis in mice as influenced by diet has been more extensively studied than any other tumor of spontaneous origin, and for that reason it seems appropriate to make it the basis for this discussion. It will be observed from Table 1 that a variety of nutritional regimens may delay, partially or completely inhibit the development of spontaneous mammary cancer in mice.

A primary effect of the various regimens listed in Table 1 was on body weight. Weanling mice placed on calorie restricted diets failed to gain weight. Adult animals lost from 10 to 45 per cent. in body weight, usually during the first few weeks of the experiment, after which time the animals were able to maintain their weight at the lower level. On the cystine deficient diet young mice were unable to gain in weight, while on the lysine deficiency the diet contained enough lysine to permit a very slow increase in body weight of the young mice, amounting to approximately 20 per cent. of the initial weight of the animals.

The effect on estrus and mammary gland development was equally striking in those experiments in which estrous cycles and mammary glands were studied. Estrus was absent in the weanling mice that failed to increase in body weight and occurred irregularly in the lysine deficient group in which there was some body weight gain. In the calorie restricted and deficient weanling animals in which estrus was absent or occurred occasionally, there was also complete or partial inhibition of mammary gland development. In the parous animals estrus was probably absent, and the mammary glands atrophied.

The most effective regimens in decreasing mammary cancer were those instituted at an early age on weanling mice and continued until death, although mice that were restricted in calories after raising one or two litters also showed a definite decrease in tumor incidence, loss in body weight and atrophy of the mammary glands.

Inhibition of estrus has been observed in rats[2] after a weight loss of only 15 per cent. The loss in weight of the mice as given (Table 1, 5, 6) with the concomitant inhibition of estrus suggests that estrogenic hormone production was one factor that may have been affected. The administration of diethylstilbestrol (Table 1, 9, 6), to a group of cystine deficient mice induced continuous estrus and stimulated development of the mammary glands. The incidence of mammary tumors was increased from 0 to 45 per cent., or to only approximately half that found in the normal controls.

[1] V. R. Potter, SCIENCE, 101: 105, 1945.

[2] M. G. Mulinos, L. Pomerantz, J. Smelser and R. Kurzrok, *Proc. Soc. Exp. Biol. and Med.*, 40: 79, 1939.

The decreased incidence or inhibition of spontaneous mammary tumors in mice by the various dietary regimens listed in Table 1 suggest that the influence of diet on mammary carcinogenesis is not a direct effect of some specific dietary constituents,[3] but is more likely due to the potentialities a dietary procedure has in altering estrogenic and probably other hormonal secretions over a considerable portion of the animal's life span. Obviously, any dietary regimen that in some way inhibits estrus or the development of the mammary gland would be expected to inhibit or delay spontaneous mammary carcinogenesis in mice.

The question arises: To what extent would the dietary regimens found effective in inhibiting mouse mammary cancer be effective in delaying or preventing the same disease in man? Even though they were unduly optimistic to conclude from any experimental evidence available at present that "eating no more than we need" would be a useful guide in decreasing or inhibiting human cancer.

It is further emphasized that the effective dietary regimens discussed herein were applied to a single type of tumor that develops spontaneously only in the females of susceptible strains of mice. That broad generalizations on the effect of nutrition on carcinogenesis should not be attempted, even in animals, from results on a single tumor type are illustrated by results reported from the McArdle Laboratory,[4] where it was observed that well-nourished rats as well as rats consuming an optimum amount of protein (18 per cent. casein) were more resistant to the development of liver tumors following the ingestion

TABLE 1

DIETARY MEASURES FOUND EFFECTIVE IN DECREASING THE INCIDENCE OF SPONTANEOUS MAMMARY CANCER AND OTHER OBSERVED EFFECTS SUCH REGIMENS HAVE ON FEMALE MICE

Strain	Age months: Started	Age months: Ended	Breeding	Type of regimen	Amount of restriction	Body weight change per cent.: Control Per cent.	Body weight change per cent.: Exp. Per cent.	Effect on: Amount of mammary gland development	Effect on: Estrus	Effect on: Tumor incidence per cent.: Control	Effect on: Tumor incidence per cent.: Exp.
dba[2]	2.5	20	Nulliparous	Calorie restriction	1/2	+50	−10	.....	.....	38	0
dba[3]	5.5	18	"	Underfeeding	1/3–1/2	+30	−<25	.....	.....	40	2
dba[3]	9.0	24	27 per cent. nulliparous 73 per cent. parous	"	1/3–1/2	+ 7	−>30	.....	.....	30	7
C3H[4]	1	18	Nulliparous	Calorie restriction	1/3	+170	+10	.....	.....	67	0
C3H[5, 6]	1	22	"	" "	1/2	+106	− 8	infantile	complete absence	100	12.5
C3H[5, 6]	4	22	Parous	" "	1/2	+33	−44	atrophy	.....	100	18.2
C3H[7, 6]	1	28	Nulliparous	Lysine deficient	.....	+100	+>20	infantile to moderate	occasional	97.6	25.0
C3H[8, 6]	1	22	"	Cystine deficient	.....	+100	0	infantile	complete absence?	97.4	0
C3H[9, 6]	1	17	"	Cystine deficient + stilbestrol	.....	+100	0	extensive	continuous	92.0	45.0

[2] A. Tannenbaum, *Cancer Res.*, 2: 460, 1942.
[3] *Idem, Am. Jour. Cancer*, 38: 335, 1940.
[4] M. B. Visscher, Z. B. Ball, R. H. Barnes and I. Sivertson, *Surgery*, 11: 48, 1942.
[5] F. R. White, J. White, G. B. Mider, M. G. Kelly and W. H. Heston, *Jour. Nat. Cancer Inst.*, 5: 43, 1944.
[6] F. R. White, *Jour. Nat. Cancer Inst.*, 5: 49, 1944.
[7] F. R. White and J. White, *Jour. Nat. Cancer Inst.*, 5: 41, 1944.
[8] J. White and H. B. Andervont, *Jour. Nat. Cancer Inst.*, 3: 449, 1943.
[9] F. R. White and J. White, *Jour. Nat. Cancer Inst.*, 4: 413, 1943.

partially efficacious, would not some of the other conditions likely to develop, such as failure of normal growth, inhibition of normal mammary gland development or the possible cessation of reproductive capacity completely negate any attempt to restrict the human diet to the extent apparently necessary to obtain prevention of cancer in the mouse? On the other hand, if a relatively small restriction of the food intake would be effective either in delaying or partially decreasing the incidence of cancer without also affecting other body functions it might be of great practical value. Such studies have not yet been carried out with experimental animals, and it seems

[3] H. P. Morris, Symposium No. 28, The American Association for the Advancement of Science, pp. 140, 1945.

of paradimethylaminoazobenzene than were rats consuming this carcinogenic hydrocarbon in nutritionally poor diets such as those containing brown rice and carrots or suboptimum amounts of protein.

An effort as mentioned by Potter[1] has been made to correlate the diet and development of cancer in man from statistics on the relation of weight and cancer incidence as obtained from life insurance records. These statistics have been brought together by Tannenbaum.[5] In general this material shows that among people who were overweight at the time of issuance of the insurance policy there was a somewhat greater

[4] J. A. Miller, M. S. Miner, M. D. Rusch and C. A. Baumann, *Cancer Research*, 1: 699, 1941.
[5] A. Tannenbaum, *Arch. Path.*, 30: 509, 1940.

proportion of deaths due to cancer than among persons not overweight at the time the policy was issued. In one insurance report the relationship between overweight and cancer was more marked for cancer of the female genital organs and intestines than for cancer of the breast, stomach, liver or gall bladder. Other statistics did not show any relationship between weight and cancer mortality. The most favorable statistics did not support this relationship very strongly and even if further work substantiates a relationship between overweight and death from cancer, one can not be certain that the relation between cancer and weight is direct; there may be several factors little understood that affect the development of obesity in an individual.

Furthermore, since these weight records were made many years prior to death from cancer it seems undesirable to place too much emphasis on them. Tannenbaum's suggestion[5] of the need of securing more data on the relationship of weight to death from cancer is a good one. Many factors, including composition and amount of diet, should be studied before drawing final conclusions or making specific recommendations.

The role of exercise upon cancer development has not been extensively investigated experimentally. Kline and Rusch[6] have recently reported the results of forced exercise on a transplanted sarcoma in mice. No difference was observed in the number of takes in exercised and non-exercised mice. It seems that if there is any phase of a tumor transplantation experiment which might be classified in the "critical period,"[6] as used by Potter,[1] it would be the period before the transferred tumor cells began to grow in their new host, yet it was only during the growth period of the transplanted sarcoma that forced exercise was observed to slow tumor growth. This would certainly come under the "period of progression" as defined by the above-mentioned authors. It is entirely possible that forced exercise may also play some role in carcinogenesis during this so-called critical period, but the evidence cited seems inadequate. It may be more nearly correct to conclude from the data that forced exercise decreases tumor growth instead of genesis of tumor. Even here such an interpretation is complicated, as Potter also points out, because of the concomitant lowered food intake of the exercised mice.

This survey of the experimental evidence on the genesis of spontaneous mammary tumors in mice leads one to conclude that dietary regimens, so far as are known, which inhibit or delay the disease in animals are too drastic even if applicable to be of practical value as a means of preventing human cancer. The effect of exercise on carcinogenesis has been too inadequately investigated, even in animals, to be used as a guide in drawing definite conclusions about its efficacy as a measure in the prevention of human cancer. On the other hand, nutritionally good diets have been observed to delay carcinogenesis. Therefore, no broad generalization on the effect of nutrition in the prevention of human cancer should be made at this time.

[6] B. E. Kline and H. P. Rusch, *Cancer Research*, 4: 762, 1944.

HAROLD P. MORRIS
NATIONAL CANCER INSTITUTE,
BETHESDA, MD.

FAT CONTENT OF GUINEA-PIG MILK

DURING a search of the literature for data on the guinea pig, I found several references which quoted the fat content of guinea-pig milk as being 45.80 per cent. This figure had been taken from page 470 of "Physiological and Pathological Chemistry" by G. Bunge (2nd Eng. Ed., 1902, Philadelphia, Blakiston). Going back to the original paper, I found that the author was Professor Purdie (Chemical Laboratory of St. Andrews) and that the paper was published in *Chemical News,* Volume 52, page 170, 1884. Actually this paper deals with dolphin milk. The editors of the above-mentioned edition of Bunge's text-book got their information from "Chemie der menschlichen Nahrungs- und Genussmittel," by Dr. J. König, which quotes Purdie's figures for "Meerschwein Milch (Delphinus phocaena)" as being 45.80 per cent. Thus a mistake has been made by translating "Meerschwein" to "guinea pig." The German for guinea pig is Meerschweinchen.

To show that Bunge himself did not believe that guinea pigs had such an excessively fat milk, I would like to refer to Table I, page 142, in his book "Physiologie des Menschen" (1905, Leipzig). Here he quotes the guinea-pig milk as having 7.1 per cent. fat and dolphin milk 43.8 per cent.

Abredhalden in "Physiological Chemistry," page 654, gives the two figures 7.31 per cent. and 6.96 per cent. for fat content of guinea-pig milk. A few samples of guinea-pig milk tested in the laboratory for experimental biology of this department gave about 6.0 per cent. fat. It is known that this figure is a little low, because the last milk was not taken from the nursing guinea pigs.

The above error in translation has resulted in a wide-spread misconception as to the fat content of guinea-pig milk, 45.80 per cent. being the figure most commonly quoted. I feel, therefore, that the matter should be brought to the attention of your readers.

V. E. ENGELBERT

TEMPERATURE TOLERANCE OF MALE GERM-PLASM

As a physicist with only an elementary knowledge of biology, my remarks on R. B. Cowles's article on "Temperature Induced Sterility" in the March 2 issue of SCIENCE may be a bit naive. It seems to me, however, that the exposed location of the testes in an environment generally lower than body temperature is a natural cause of the lack of tolerance of male germ-plasm for high temperatures. It might be considered significant that the ovaries do not have an exposed location and do not have a low temperature tolerance. The causal chain would seem to be (1) exposed location of the testes to facilitate the reproductive act; (2) low temperature tolerance of male germ-plasm resulting from exposed location, rather than, as Cowles implies, (1) low temperature tolerance of male germ-plasm, and (2) location of plasm in a region kept below normal body heat.

WILLIAM V. SMITH
MASSACHUSETTS INSTITUTE OF TECHNOLOGY

TRANSLITERATION OF RUSSIAN

I HAVE been reading with a great deal of interest the discussion on the transliteration of Kyrillic for English-speaking people. I think the growing importance of Russian technical literature makes finding a good solution to this problem imperative.

A satisfactory transliteration should fulfil the three following requirements: (1) When pronounced as an English word, reproduce fairly closely the sound of the Russian word; (2) should not require unusual (out-of-font) characters; (3) should allow unique reconstruction of the word in Kyrillic characters, so that it might be looked up in a Russian-English dictionary.

The *Chemical Abstracts* transliteration meets the first two requirements very well; I believe that the following simple modifications which are also in accord with the first two requirements given above, would enable it to meet the third requirement.

The modifications suggested are these:

(1) The soft sign should be represented by an apostrophe both in the middle and at the end of a word. If it is omitted at the end of a word, one will look for the word in the wrong place in the dictionary.

(2) The hard sign is not used much any more—never at the end of a word. To distinguish it from the soft sign, it could be represented by two apostrophes (''). Thus the verb "to explain" would be written ob''yasnyat'.

(3) E should be translated "ye." The character ë should be added to the list of Kyrillic characters, to be transliterated "yo." (All accented "e's" should not be transliterated "yo"—as this would cause too much confusion in reconstructing the Kyrillic word. Only e's specifically marked ë in the Russian text should be so treated.)

(4) In order to render the reconstruction of the Kyrillic word unique, the transliteration of bI as "y" could not be retained. Perhaps ü would be as good as any—and certainly as much in accord with requirement (1) as "y" is at present.

It is freely admitted that defects remain in this system, such as that of treating the genitive singular ending of adjectives and pronouns. However, I believe that the system I propose would enable any one moderately familiar with Russian to construct correctly the original Kyrillic work from its transliterated equivalent, thus meeting requirement (3). Of course, this entire argument applies only to the new orthography, for which I believe the modified transliteration permits close observance of my third requirement, without disturbing significantly the advantages of the *Chemical Abstracts* system in regard to the first two requirements.[1]

JAMES B. HICKMAN
CHARLESTON, W. VA.

SCIENTIFIC BOOKS

AGRICULTURAL CHEMISTRY

A Source Book of Agricultural Chemistry. By CHARLES A. BROWNE. x + 290 pp. 32 figures. Waltham, Mass.: Chronica Botanica Company. 1944. $5.00.

DR. BROWNE'S "Source Book of Agricultural Chemistry" has a far broader appeal than is implied in its title; it is a thoroughly scholarly document in the history of chemistry. Agricultural chemistry is understood by the author to mean the applied science that deals with "the chemical composition and mutual chemical relations of soils, fertilizers, crops, and farm animals in so far as they concern the production upon the farm of agricultural supplies." Accordingly, not only is the fundamental chemistry of the elements and their compounds involved but also many aspects of biochemistry, physiology, botany, geology, and even meteorology and engineering. Thus the book includes discussions among many others of such men as Paracelsus, Bacon, Boyle, Stahl, Priestley, Cavendish, Scheele, Lavoisier, Davy, Mulder and Liebig, whose work laid the early experimental and theoretical foundation of pure chemistry and who must be considered in any historical introduction to this science, together with Grew, Hales, Ingen-Housz, de Saussure,

[1] The transliteration of all characters not mentioned above would remain as now indicated in *Chemical Abstracts.*

e Candolle and Boussingault, who were the most important of the early workers in the physiology of lants, and, in addition, Tull, Duhamel, Home, Thaer, haptal, Schübler and Sprengel, who were the leaders the application to the problems of agriculture of the bservations and speculations of these other investiators.

The book contains seven chapters in which the men hiefly responsible for the progress of agricultural hemistry are treated in historical order beginning ith an entertaining and highly informative account f the theoretical views and accomplishments of the reeks and Romans. This is followed by a discussion f representatives of the sixteenth and seventeenth entury alchemical and iatrochemical schools of hought, and by successive chapters on the workers f the period of the early Royal Society, of the early nd the late phlogiston period, of the period of the hemical revolution that began with Lavoisier, and y a final one upon more recent investigators up to he time of Liebig. It is within this framework that he author describes the conditions which led to the pplication to agricultural science of modern scienific views, a result for which Liebig was in many ways rimarily responsible through the publication in 1840 f his celebrated book, "Organic Chemistry and Its Applications to Agriculture and Physiology."

Nothing so comprehensive and at the same time so letailed has, to the knowledge of the reviewer, been ttempted and so satisfactorily accomplished. Although the book is far indeed from being light reading, it is invariably interesting and stimulating; the product of a ripe scholarship and of an amazing breadth of knowledge and experience, it fills a place in the history of chemical science that has hitherto been only superficially if at all dealt with. It will doubtless become required reading in all courses that attempt to describe this field.

The text contains biographical sketches which are illustrated by comprehensive quotations from the writings of the investigators discussed, and illuminated by clear descriptions of the fundamental experiments upon which their most important contributions were based. The numerous figures include reproductions of the title pages of many of the classical publications in chemistry and in agriculture, as well as illustrations of apparatus or experiments taken from the originals. Each of the sections contains a selection of references to the titles of the more important books and papers of the subject as well as to other biographical and historical material concerning him or his work. The book ends with an appendix which gives an outline of sources in the history of agricultural chemistry from the time of Liebig to the present. For this period there is a wealth of material available in any library; what Dr. Browne has accomplished is to make the earlier and rarer material available in brief and attractive form by publishing what is in effect an abstract of a lifetime of reading and research into the background of his field of special interest.

H. B. VICKERY

CONNECTICUT AGRICULTURAL EXPERIMENT STATION,
NEW HAVEN, CONN.

THE IMPROVEMENT OF LIVESTOCK

Livestock Improvement. By J. E. NICHOLS. 208 pp. Illustrated. Edinburgh: Oliver and Boyd, Ltd. 1944. Price 10/6.

THE excellent little volume is a valuable addition to the literature on animal breeding. The author has brought together a wealth of material, drawn from a broad experience and extensive travel. The presentation of the genetic background for problems of animal breeding is sound and clear. The author may have gone a little too far in his emphasis of the influence of the environment, but this emphasis does have the merit of counteracting the tendency of some texts on breeding to ignore entirely this factor. Much consideration was properly given to the problem of acquiring adequate standards for measuring genotypic differences among animals. This text is not designed for the novice, since there has been no avoidance of technical terms or some of the involved phases of selection. Certain topics such as crossbreeding have been dealt with somewhat arbitrarily, but this may be expected in a text so limited in scope.

D. C. WARREN

KANSAS STATE COLLEGE

REPORTS

CONFERENCE ON SCIENCE EDUCATION FOR NATIONAL SECURITY

A CONFERENCE on "Science Education for National Security," held at the Engineers Club in Philadelphia on Friday, March 30, 1945, was attended by 50 representatives of science, industry and labor.

The leading speaker at the meeting was Dr. Marion H. Trytten, director of scientific personnel of the National Research Council, who reported on pending and proposed legislation designed to alleviate the alarming scarcity of highly trained scientific workers and on other steps being taken to improve the situation. Dr. Trytten predicted that the termination of hostilities would bring little relief because demand

for scientific personnel will continue to outstrip the supply. Military research will continue and universities must enlarge their faculties to fill the gap in training caused by the wartime lag; they are witnessing the beginning of an influx of foreign students. The demand by foreign countries for scientific and technical personnel is expected to be very great.

Despairing of bringing about a reversal of the administrative policy that has been responsible for the excessive drafting of irreplaceable personnel, leaders of science are turning their effort to legislative methods, Dr. Trytten reports. While most of the damage has already been done it is imperative that training in the physical sciences particularly be resumed immediately. Legislation in preparation would select 20,000 men a year for such training.

Dr. Reuben T. Shaw, chairman of the National Science Teachers Association's Committee on Public Relations, presided at the meeting. Dr. Philip G. Johnson, president of the association, described science education's job as two-fold: to supplant widespread scientific illiteracy with a basic scientific culture and to train technical workers for science and industry. He suggested that industrial advertisers who have been saluting the role of the teacher in education should call attention to the special importance of the science teacher.

Dr. Ivor Griffith, president, and Dr. Rachel Anderson, vice-president, of the Middle States Science Teachers Association, were among the speakers. Dr. Griffith, who also is president of the Philadelphia College of Pharmacy and Science, paid special tribute to the pharmaceutical industry for pre-war research that made possible the remarkable wartime developments in medicine, and for that industry's generous and timely support of science education.

The spokesman for industry was E. C. Fuller, president of the Curtis Publishing Company and former president of the National Association of Manufacturers. Mr. Fuller reported on surveys made by the NAM which clearly showed industry's large stake in better education. He cited the particular need for a public with an understanding of scientific principles.

Joseph F. Burke, educational director of the American Federation of Labor, spoke of labor's strong support for better educational opportunities. Also present was W. B. Woodward, Jr., of the Brotherhood of Locomotive Firemen and Enginemen.

Dr. Homer C. Will, president of the Pennsylvania Academy of Science; Dr. Bradford Willard, president-elect, and several members of the executive committee attended the meeting as well as Dr. Walter S. Lapp, president of the Philadelphia Science Teachers Association, and members of its executive committee.

Dr. William A. Feirer, vice-president of Sharp and Dohme; Franklyn Waltman, public relations director of the Sun Oil Company; Roy Stryker, George Freyermuth and Barry Meglaughlin, of the Standard Oil Company of New Jersey; J. A. Lorimer and H. J. Drucquer, of the Standard Oil Company of Pennsylvania, and H. R. Clark and A. F. Natters, of the Radio Corporation of America; James K. Hunt, public relations director of E. I. du Pont de Nemours and Company; and P. L. Schauble, vice-president (public relations) of the Bell Telephone Company of Pennsylvania, and W. M. Welch also were present.

Dr. Irving P. Reimann, director of the Lankenau Hospital Research Institute, represented the American Medical Association and spoke of the critical need for more science in the secondary school in the interest of public health. The American Legion was represented by Dr. Charles E. Sohl, the American Chemical Society by Dr. F. T. Tyson, Sigma Xi by Dr. James A. Harrison, the Physics Club of Philadelphia by Dr. M. R. Wehr, and various engineering societies by Dean J. H. Billings, of the Drexel Institute of Technology, Philip H. Spear and F. V. Westermaier.

SPECIAL ARTICLES

IN VITRO EVIDENCE OF REGENERATION OF ACTIVE PENICILLIN FROM PENICILLIN ESTERS

INDIRECT evidence for the existence of bacteriostatically inactive penicillin esters and their ability to hydrolyze to yield bacteriostatically active penicillin has been presented by Meyer, Hobby and coworkers.[1, 2] Their esters were prepared by the reaction of a diazoalkane with the acid form of penicillin in chloroform. Evidence for the regeneration of bacteriostatically active penicillin from the esters was deduced from experiments *in vivo*. These showed that mice inoculated with lethal doses of a hemolytic streptococcus were protected by oral or subcutaneous administration of solutions of the ethyl or n-butyl esters.

Direct evidence has been found which shows that penicillin esters may be converted to active penicillin *in vitro*.

The methyl ester of penicillin was prepared by addition of diazomethane[3] to 250 ml of an amyl acetate solution of the free acid of penicillin which assayed initially 5,990 International Units per ml. After re-

[1] K. Meyer, G. L. Hobby and E. Chaffee, SCIENCE, 97: 205–206, 1943.

[2] K. Meyer, G. L. Hobby and M. M. Dawson, *Proc. Soc. Exp. Biol. and Med.*, 53: 100–104, 1943.

igerating for 24 hours, a cup plate assay against *taphylococcus aureus* A.T.C.C. No. 6538 showed 12 U. per ml. After washing with 750 ml of 1 per nt. $NaHCO_3$ buffer adjusted to pH 7.8 followed by 0 ml of water, the assay of the amyl acetate solution s 30 I.U. per ml. The combined wash liquid as-yed less than 5 I.U. per ml.

The resulting washed amyl acetate solution was con-ntrated at 30° C under vacuum to a yellow oil eighing 1.46 g. Theoretically, this oil should have a otential potency of about 1,000 I.U. per mg. It was ssolved in dry methanol to form 30 ml of solution. he theoretical potential potency of the solution was out 50,000 I.U. per ml.

Emulsions were prepared by adding the methanol lution of the ester to distilled water to which were ded sodium bicarbonate and Duponol[4] as indicated Table 1. These mixtures were held at 5° C and re assayed at intervals with the results as indicated Table 1. The fact that the methyl ester of peni-llin can be hydrolyzed *in vitro* to yield active peni-llin is evident.

The ethyl ester of penicillin was prepared in the me manner as the preparation of the methyl ester, cept that diazoethane[3] was employed as the esteri-ing agent and the yellow oil concentrate was dis-lved in dry ethanol to make 30 ml of solution. This so should have a theoretical potential potency of out 50,000 I.U. per ml.

Suspensions of the ethyl ester were prepared and ndled in the same manner as were those of the

TABLE 1

REGENERATION OF ACTIVE PENICILLIN FROM THE METHYL ESTER

Emulsion*	$NaHCO_3$ added mg	Duponol added mg	pH	Assay, I.U. per ml Day: 0	1	2	3	4	Maximum reactivation per cent. of theory
1	0	0	6.0	12	30	17	17	14	4.8
2	20	0	7.3	13	136	161	150	114	25.8
3	0	5	5.8	14	3	1	0.2	.2	2.2
4	20	5	7.1	18	73	119	117	105	19.0

*All emulsions were prepared by adding 0.25 ml of the ethanol solution of the methyl ester to 19.75 ml of dis-led water. The theoretical potential potency of the ulsion was about 625 I.U. per ml.

ethyl ester. The fact that active penicillin may be generated by hydrolysis of this ester is shown in ble 2.

The emulsions were physically stable in the pres-ce of Duponol or $NaHCO_3$ or both, but in the ab-nce of these agents (emulsions 1 and 5) some of the y material collected on the walls of the containing

[3] C. R. Noller, "Organic Syntheses," Vol. XV, pp. 3–5, ew York: John Wiley and Sons, Inc. 1935. Diazoe-ane was prepared by the same general method.

TABLE 2

REGENERATION OF ACTIVE PENICILLIN FROM THE ETHYL ESTER

Emulsion*	$NaHCO_3$ added mg	Duponol added mg	pH	Assay, I.U. per ml Day: 0	1	2	3	4	Maximum reactivation per cent. of theory
5	0	0	6.2	7	6	8	7	6	1.3
6	20	0	7.2	10	34	98	100	66	16.0
7	0	5	6.0	9	1	0.3	.2	.2	1.4
8	20	5	7.3	10	33	59	55	42	9.5

*All emulsions were prepared by adding 0.25 ml of the ethanol solution of the ethyl ester to 19.75 ml of distilled water. The theoretical potential potency of the emulsion was about 625 I.U. per ml.

vessels. The emulsions became increasingly transparent on standing, particularly in the presence of $NaHCO_3$. Hydrolysis was indicated. Duponol[4] appears to exert an inactivating effect on penicillin.

Both the methyl and ethyl esters are slightly soluble in water with the methyl ester being the more soluble of the two. Data in Tables 1 and 2 show that the methyl ester is also the more readily hydrolyzed of the two esters. Methyl esters are generally formed and hydrolyzed more readily than esters of higher alcohols.[5]

Although the theoretical amount of active penicillin has not as yet been obtained from these bacteriostatically inactive esters, the fact that a significant fraction may be regenerated *in vitro* is of interest.

Summary: It has been found that methyl and ethyl esters of penicillin may be hydrolyzed *in vitro* to yield 26 and 16 per cent. respectively of the theoretical bacteriostatically active penicillin.

RICHARD J. HICKEY

RESEARCH LABORATORIES,
COMMERCIAL SOLVENTS CORPORATION,
TERRE HAUTE, IND.

THE EFFECT OF ULTRAVIOLET RADIATION ON CYSTS OF ENDAMOEBA HISTOLYTICA[1]

THE object of these experiments was to determine whether or not ultraviolet radiation is lethal to the cysts of the intestinal pathogen, *Endamoeba histolytica.*

Two models of ultraviolet lamps were supplied by the General Electric Company. Both lamps emitted energy of wave-length 2,537A, which lies within the region where the maximum germicidal effect might be expected.[2] Distilled water suspensions of the cysts,

[4] Duponol ME, E. I. du Pont de Nemours and Co., Wilmington, Del.

[5] A. Michael and K. Wolgast, *Ber.*, 42: 3157–3176, 1909.

[1] The material in this article should be construed only as the personal opinion of the writers and not as representing the opinion of the U. S. Navy Department.

in concentrations ranging from 30,000 to 200,000 cysts per liter, were irradiated for various periods of time. Following irradiation, the suspensions were sedimented and the residue planted upon a suitable culture medium. Subsequently, the cultures were examined for the presence of motile trophozoites. A control suspension was run with each test, and handled in the same manner except that irradiation was omitted.

It was found that all *E. histolytica* cysts were destroyed by ten minutes' irradiation in the five tests done upon suspensions of 30,000 cysts per liter of distilled water (Tables 1 and 2). Heavier suspensions were not tested for this time interval, since 30,000 cysts per liter exceeds the concentration likely to be encountered in water purification practice. When heavier suspensions and shorter exposures were used, only a portion of the cysts were destroyed (Tables 1 and 2). The extreme sluggishness and

TABLE 1

CYSTICIDAL EFFECT OF IRRADIATION WITH GERMICIDAL LAMP, MODEL 1

Test (number)	Exposure time (minutes)	*E. histolytica* cysts per liter of distilled water	Results per cent. survival*	
			Test	Control
1	1	200,000	0	100
2	2	200,000	20	100
3	3	200,000	0	100
4	4	200,000	20	100
5	5	100,000	0	100
6	1	58,000	0	100
7	2	58,000	0	100
8	3	58,000	60	100
9	4	58,000	20	100
10	10	30,000	0	100
11	10	30,000	0	100
12	10	30,000	0	100
13	10	30,000	0	100

* Per cent. survival is the ratio of the number of positive cultures to the total number of culture tubes inoculated with the sediment from the cyst suspension.

TABLE 2

CYSTICIDAL EFFECT OF IRRADIATION WITH GERMICIDAL LAMP, MODEL 2

Test (number)	Exposure time (minutes)	*E. histolytica* cysts per 4 liters of distilled water	Results per cent. survival*	
			Test	Control
1	5	216,000	10	100
2	5	200,000	0	100
3	5	120,000	10	100
4	10	120,000	0	100

* Per cent. survival is the ratio of the number of positive cultures to the total number of culture tubes inoculated with the sediment from the cyst suspension.

altered general appearance of the trophozoites which did emerge from the cysts surviving exposure indicated a sublethal effect of ultraviolet radiation upon these organisms; such trophozoites changed shape but slowly and showed none of the explosive pseudopod formation typical of *E. histolytica*. The control cultures showed normally active trophozoites.

Although ozone is produced while the lamps are in use, the amount is small and the effect apparently negligible.[3, 4]

ALICE M. STOLL,
Ensign, W(H), USNR
PAUL A. WARD,
Ensign, D(L), USNR
DON R. MATHIESON,
Lieutenant, MC(G), USNR

INSECTICIDAL ACTIVITY OF SOME ALKOXY ANALOGS OF DDT

SEVERAL 2,2-bis(*p*-alkoxyphenyl)-1,1,1-trichloroethanes were synthesized by the general method described[1] for the ethoxy member and were tested in comparison with DDT, 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane (m.p. 107°).

The results of the tests on houseflies (*Musca domestica* L.), which were obtained by the large Peet-Grady method,[2] are shown in Table 1. Deo-Base, a refined petroleum distillate, was the solvent used for making the spray solutions. The percentage kill given by the Official Test Insecticide (OTI) was determined for each culture of flies to provide an index of the resistance of the culture.

TABLE 1

TOXICITY OF SOME *Para*-SUBSTITUTED DIPHENYLTRICHLOROETHANES OF THE GENERAL FORMULA $(R\text{–}C_6H_4)_2CHCCl_3$ TOWARD HOUSEFLIES AS DETERMINED BY THE PEET-GRADY METHOD

Substituent R is:	Concn. of compound g/100 ml	Pyrethins added g/100 ml	Knock-down in 10 min., per cent.	Kill in 24 hrs., per cent.	OTI kill in 24 hrs., per cent.
Cl (DDT)	.25	..	42	38	49
CH_3–O (Methoxy)	.15	..	91	31	51
" "	.20	..	99	32	34
" "	.40	..	99	85	46
C_2H_5–O (Ethoxy)	.20	..	90	86	34
" "	.10	.05	97	78	44
" "	.15	.05	99	91	44
Cl (DDT)	.10	.05	96	86	44
Control	..	.05	96	34	44
C_2H_5–O + CH_3–O	.15 + .15	..	97	88	44
Cl (DDT) + CH_3–O	.10 + .15	..	94	86	44
n–C_3H_7–O (*n*-propoxy)	.50	.025	95	48	54
n–C_4H_9–O (*n*-butoxy)	.50	.025	99	25	54

With DDT used at a concentration of .25 g per 100 ml the knock-down in 10 minutes was very poor, but most of the flies knocked down were dead in 24 hours.

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[3] H. S. Forbes and G. A. Daland, *Am. Jour. Physiol.* 66: 50, 1923.

[4] L. R. Koller, *Jour. Applied Physics*, 10: 9, 630, September, 1939.

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The fate of the flies not knocked down was undetermined. Slow knock-down is one of the recognized shortcomings of DDT.[3]

The methoxy analog, 2,2-di-*p*-anisyl-1,1,1-trichloroethane (m.p. 89°), showed a surprisingly good knock-down; with the use of .15 g per 100 ml it was not quite up to the required standard, but with .2 g per 100 ml it was very satisfactory. These lower concentrations gave poor kills, but .4 g per 100 ml gave a good kill. Other investigators [4,5,6] have indicated that this DDT analog shows insecticidal activity.

The ethoxy analog, 2,2-di-*p*-phenetyl-1,1,1-trichloroethane (m.p. 105°), at a concentration of .2 g per 100 ml gave a good kill and a knock-down distinctly better than that given by DDT although not as good as that given by the methoxy analog. In order to compare the ethoxy analog with DDT more adequately, pyrethrum extract was added to the spray solutions to provide a satisfactory knock-down. The results of these runs show that the ethoxy analog may be at least two-thirds as effective as DDT against flies. By adding .15 g of the methoxy analog per 100 ml of the solutions of these compounds to provide the knock-down, this relative effectiveness was again attained.

The *n*-propoxy analog (m.p. 62°) showed a low order of toxicity toward houseflies and the *n*-butoxy analog (m.p. 50°) practically no toxicity.

DDT, the methoxy and the ethoxy analogs were found about equally effective against mosquito larvae (*Culex quinquefasciatus* Say); concentrations of .03 to .04 parts per million (p.p.m.) in tap water killed half of the larvae in 20 hours. The *n*-propoxy analog at .4 p.p.m. gave about a 50 per cent. kill while the *n*-butoxy analog at 4 p.p.m. gave a negligible kill.

Preliminary feeding tests were conducted to determine the comparative toxicity to white rats of the ethoxy analog and DDT, since the latter substance is known to be toxic[7] to higher animals and the ethoxy compound has shown excellent insecticidal activity. Four animals, about four months old, two males weighing about 320 g each and two females weighing about 200 g each, were used for each compound and for the control. The ration consisted of ground Rockland Rat Diet plus 2 per cent. corn oil. The test compound in each case was uniformly distributed in the mixture at a level of .2 per cent. All the rats receiving DDT soon developed severe tremors and died within 8 to 10 days. The female rats receiving the ethoxy analog developed tremors somewhat later and died, one after 15 and the other after 21 days. The males receiving the ethoxy analog were still apparently normal after four weeks. Autopsies did not reveal any gross pathologic changes attributable to the ethoxy analog. The results suggest that the ethoxy analog may be less toxic than DDT to higher animals.

EDWARD A. PRILL
ALBERT HARTZELL
JOHN M. ARTHUR

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.,
YONKERS 3, N. Y.

[3] W. A. Gersdorff and E. R. McGovran, *Jour. Econ. Ent.*, 37: 137, 1944.
[4] P. Läuger, H. Martin and P. Müller, *Helv. Chim. Acta*, 27: 892, 1944.
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CRYSTAL STRUCTURE OF DDT [2,2-bis (p-CHLOROPHENYL) 1,1,1,-TRICHLOROETHANE]

A SAMPLE of DDT (m.p. 106° C.) recrystallized several times from mixtures of ethanol and water was chosen for this study. With the fine hair-like needles, rotation, oscillation, Weissenberg and powder x-ray diffraction patterns were made and reciprocal lattice projections used to index interferences and to check interpretations of results.

The material appears to crystallize in the orthorhombic system with unit cell dimensions

$a_0 = 19.25$ A.U.
$b_0 = 10.04$ A.U.
c_0 (along needle axis) $= 7.73$ A.U.

Calculations from density measurements (approximately 1.0) indicate two molecules per unit cell, and the space group is probably $P222_1$. Further work on the molecular configuration is now in progress together with efforts to produce crystals of greater cross section in order to improve rotation and Weissenberg patterns around a and b axes. For purposes

TABLE 1

Line No.	d_{hkl} (Å. U.)	Planar indices	Relative intensity
1	9.50	200	0.5
2	5.90	311	0.89
3	5.45	310	0.28
4	5.00	020	1
5	4.36	411	0.70
6	4.17	221	0.77
7	3.85	002	0.34
8	3.57	420	0.49
9	3.36	421	0.47
10	3.05	600 (230) (031)	0.49
11	2.93	231	0.37
12	2.82	330	0.37
13	2.75	700	very faint
14	2.59	003	" "
15	2.51	040	" "
16	2.40	240	" "
17	2.31	340	" "
18	2.26	430 (041)	" "
19	2.20		" "
20	2.06		" "
21	2.01	[004]?	" "
22	1.74		" "

of identification, powder diffraction interferences are given in Table 1.

G. L. CLARK
F. W. CAGLE, JR.

NOYES CHEMICAL LABORATORY,
UNIVERSITY OF ILLINOIS

OBSERVATION OF BACTERIOPHAGE THROUGH A LIGHT MICROSCOPE[1]

ELECTRON micrographs of bacteriophage attacking *Escherichia coli*,[2] *Salmonella pullorum*[3] and *Rhizobium leguminosarum*[4] show that the particles have a diameter greater than that of bacterial flagella; with the latter organism, the particles have a diameter 5 to 10 times greater than that of flagella. Since the bacterial flagella can be stained and made visible under an ordinary light microscope, it seemed that the same should be possible with bacteriophage particles (sometimes called bacterial viruses).[2]

The discovery was made during the winter of 1943–44 that bacteriophage preparations treated with auramin and observed under the microscope when radiated with ultraviolet rays from an H-4 mercury arc show the presence of many bright yellow pinpoints of light in an otherwise dark field. Bacteria failed to show anything other than a very pale and weak yellow color, and the granules were not observed unless bacteriophage particles were known to be present. The past winter this work was repeated with quite similar results, and two additional methods for revealing bacteriophage particles were developed. These involved the use of stains (one, a modification of the acid-fast stain) and ordinary light; and by these the bacteriophage particles were seen to have the same shape as under the electron microscope.

Since at that time the bacteriophage had not been studied in active condition, there was no way of being absolutely certain as to the sequence in the process of lysis of the various structures. The drawings presented in Fig. 1, however, show the apparent order of events. Beginning with bacterial cells of the pea nodule organism (*a*), which show the refractile bodies usually seen following light staining with methylene blue or rose bengal, and the bacteriophage (*b*), which always stained heavily, it seemed that the first contact of the bacteriophage with the cell resulted in a delicate connection between the two, as shown in the first drawing in (*c*), the particle appearing at a greater distance from the cell than would ordinarily be expected. Other cells were seen in which the bacteriophage particle had developed to larger size and in which there had been a corresponding shortening of the connection to the bacterium, and still other cells to which relatively large particles of bacteriophage were attached.

Still other cells were observed in which these phenomena were absent (*d*), but in which the usual refractile bodies were replaced by other particles which stained heavily and had a quite different position in the cell. Other cells had apparently broken down, leaving a faintly colored material which stained in a manner characteristic of cell protoplasm, but the material was quite different in outline from the original cell and included varying numbers of deeply stained bodies. In some such partially destroyed cells, the latter bodies were small, and in others, relatively large. Next in the process of lysis it seemed that the typical structures consisted of faintly staining rem-

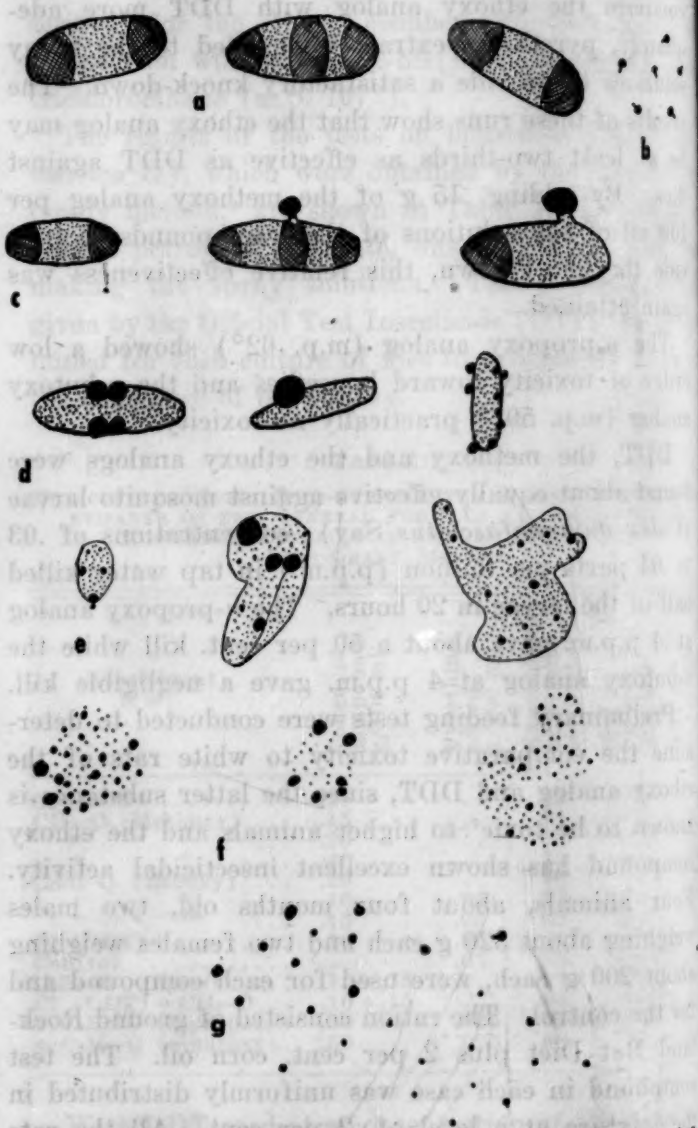

FIG. 1. Progressive stages in the lysis of bacterial cells as indicated by stained material examined under the ordinary microscope. a. Cells of the pea nodule organism. b. Bacteriophage particles. c. Cells in early stages of bacteriophage action. d. Cells in which bacteriophage action was well under way. e. Partially lysed cells showing protoplasm in which the development of densely staining bodies is evident. f. Remnants of cell protoplasm among which are numerous bacteriophage particles that have developed from the cell protoplasm. g. A concentration of densely staining bodies around an area that was probably one or more cells, but in which no cell material is evident.

[1] Jour. Paper No. 623, N. Y. S. Agric. Exp. Station, Geneva, N. Y., March 28, 1945.

[2] S. E. Luria, M. Delbrück and T. F. Anderson, *Jour. Bact.*, 46: 57–76, 1943.

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[4] A. W. Hofer. Unpublished data.

nants of cells (*f*), among which were numerous minute bodies with an intense color. In some areas (*g*) only the latter bodies were seen, the cell material apparently having disappeared. In general, the material in which the process of lysis was near completion stained easily and gave clear-cut results, while particles stained during the early stages of lysis were hard to detect; it is possible that the bacteriophage particles lost their usual staining qualities when in contact with the cell.

The difficulty involved in deciphering the exact sequence of events was largely overcome by use of the new phase difference microscope.[5] Under this, not only were structures noted as above, but the process of lysis was observed, making the details sufficiently certain that the above sequence could be completed from the stained material. The first steps in the process of lysis were followed most easily, difficulty occurring only in the observation of the last phases (due to the difficulty of maintaining the preparation on a slide). Yet, enough was seen to indicate that the process of lysis by bacteriophage may not be a well-defined, simple series of steps, but rather a group of phenomena of variable nature. Even in the study of two strains of the pea nodule organism and two races of bacteriophage specific for them, a number of unexpected developments were observed.

Beginning with the cells, which were motile and uniformly colorless, and the bacteriophage, which occurred as minute, highly refractile bluish particles, the placing of both in the same suspension bring about one or more of the following results, though so far not all have occurred in any one suspension:

(1) Loss of motility of the cells within 1 to 2 minutes after addition of the bacteriophage.

(2) Clumping of the cells, as by agglutination.

(3) Development of a blue color in the cells, apparently due to a change in the refractive index.

(4) A tendency for the cells to assume a vertical instead of a horizontal position, as if one end of the cell were heavier than the other.

(5) The uniting of a bacteriophage particle with a cell, the anchoring of the tail of the particle to the cell, the lengthening of the tail as shown by the first drawing in (c), the movement of the head of the particle with Brownian movement while the tail remained solidly anchored to the cell, the enlargement of the entire particle and the eventual rupture of the cell, with loss of cell material to such an extent that it eventually surrounded and concealed the original bacteriophage particle.

(6) The development within a cell of relatively large refractile bodies as in (d).

(7) The appearance in suspensions of lysed bacteria of many pearly white masses of cell protoplasm, each containing highly refractile bodies as in (e). Under the phase difference microscope the unstained cell material had a pearly white color, amid which developed blue bodies of two or three different sizes.

(8) Cells that were almost completely lyzed as in (f) (groups of single bodies as in (g) were not seen, as these would tend to become scattered in the suspension, even though no motility of the bacteriophage was noted).

(9) Many cells that had developed differently from what was described above, there being little or no evidence of the presence of bacteriophage of the shape indicated by the electron microscope. Such cells assume grotesque shapes, develop greatly in size, and it is believed that eventually they disappear quite suddenly.

(10) Occasional large bodies of what appears to be cell protoplasm containing small blue bodies so arranged as to be suggestive of chromatin-like material.

Thus, the evidence from the electron microscope, the stained preparations and the phase difference microscope is similar as regards the first and last stages of lysis, and the two latter agree quite well in what they show of the phenomena occurring between these points. Further evidence to the same effect is furnished by the dark-field motion picture made by Dr. A. J. Pijper, of Pretoria, South Africa, and just made available through the courtesy of Dr. Harry E. Morton, of Philadelphia.

This shows lysis by relatively large particles of a bacteriophage specific for and acting upon a nonmotile strain of *Eberthella typhosa*. While the attachment of the particles to the cells does not show as clearly as some other parts of the process, the development of the bacteriophage bodies within the cell is striking. Instead of oozing cell material, however, these cells collapsed or produced "bubbles" in the motion pictures. Thus, the interpretation of the process developed by the present authors, as it occurs with *Rhizobium leguminosarum*, is essentially similar to what was later seen in the film of *Eberthella typhosa*. Such differences as occur between the two observations are possibly due to the fact that the bacteria and bacteriophage used in the two cases were different.

Because the results of the four different methods of observation are so similar, it seems well to present this preliminary report. It is also felt that special mention should be made of the advantages of phase difference microscopy in the study of such minute bodies in active condition. Photographs taken of such material under the light microscope, procedures used in the staining of bacteriophage, description of some of the pitfalls awaiting the newcomer in the field and

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other detailed information will be published as soon as conditions permit.[6]

ALVIN W. HOFER
NEW YORK STATE AGRICULTURAL EXPERIMENTAL STATION,
GENEVA

OSCAR W. RICHARDS
RESEARCH DIVISION,
SPENCER LENS COMPANY,
BUFFALO, N. Y.

EFFECT OF ALLOXAN DIABETES ON THE GROWING RAT

THE purpose of this present investigation was to study alloxan diabetes in the immature animal and to observe the effect of early insulin deprivation on somatic growth. The effect of alloxan in destroying the β-cells of the Langerhans islets[1-5] provides a convenient method of producing diabetes experimentally. It is difficult to ascertain to what extent this metabolic disturbance affects growth in man, since most juvenile diabetics have already attained an excessive height for their age at the time the diabetes becomes apparent.

The animals used for this experiment were 26-day-old Sprague Dawley rats on a diet of Wayne Fox Chow Blox. They were divided into four groups, each consisting of four males and four females. One group was not injected and served as control for the other three groups, which were injected subcutaneously with a 1 per cent. solution of alloxan monohydrate in doses of 25, 30 and 40 mg per gm of body weight, respectively. Within half an hour after injection, alloxan appeared in the urine. It was accompanied by a fall in pH and by the red color which alloxan produces in the presence of amino acids. To control the hypoglycemia[6] which develops after the initial hyperglycemia, the rats were given a 5 per cent. glucose solution to drink for the first day after injection. Insulin was at no time administered to these animals. The alloxan treated rats except one of the females injected with the lowest dose developed diabetes. Glycosuria was more severe in the group with the largest dose and the diabetes persisted in this group, although it disappeared in several of the animals with smaller doses. All the animals in this experiment were lively after the first day and survived until sacrificed at 61 days of age or at some later date.

The effect of alloxan diabetes in stunting the growth of the rat is apparent from the figures in Table 1.

TABLE 1

Dose of alloxan mg/100 g	Sex	Body weight in grams at the age of: 26 days	35 days	42 days	48 days	55 days	61 days
Control	F	46	74	104	121	144	158 (144–168)*
25	F	45	64	94	112	131	144 (136–153)
30	F	45	67	98	115	124	149 (142–155)
40	F	47	57	70	78	95	106 (55–138)
Control	M	46	74	103	126	163	191 (180–213)
25	M	50	72	100	116	143	164 (131–192)
30	M	46	74	109	124	147	179 (169–195)
40	M	47	56	64	70	76	87 (58–111)

* Figures in parenthesis indicate range of values.

Certain other differences were observed in the diabetic rats. One out of eight animals, 0/8, and 3/8 of the 25 mg, 30 mg and 40 mg groups respectively developed cataracts of the lenses.[7] The dwarfed animals had distended abdomens, and at autopsy the stomach and intestines of these animals measured longer, relative to their body weight, than those of the controls. Some of these rats also showed infantile primary and secondary sex organs.

Summary: Severe alloxan diabetes dwarfed the immature rat.

ANNETTE CHESLER
R. TISLOWITZ
BIOLOGY LABORATORY,
SCHERING CORPORATION,
BLOOMFIELD, N. J.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SIMPLE, RAPID TECHNIC OF PREPARING WATER-IN-OIL EMULSIONS OF PENICILLIN, DRUGS AND BIOLOGICS[1]

WATER-IN-OIL emulsions have proved of value in experimental immunization procedures[2] and may become applicable to the immunization of man and domestic animals. Strauch[3] described the use of

[6] The authors wish to acknowledge helpful suggestions in regard to this work by H. J. Conn and Mary A. Darrow; the senior author wishes to express his gratitude to A. H. Bennett for the use of experimental equipment.

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ter-in-oil emulsions as a means of prolonging the ect of various therapeutic agents, but this method s received little, if any, attention.

It was found recently[4] that the intramuscular in- tion of water-in-oil emulsions of penicillin results prolongation of penicillin effect as compared with ilar amounts of penicillin injected in aqueous solu- n by the same route. It was also shown that a gle simultaneous injection of 100,000 Oxford units penicillin in water-in-oil emulsion and 50,000 units aqueous solution cured all of 40 patients with acute nococcal infection so treated.

Previous methods of preparing water-in-oil emul- ons, although simple procedures in the bacteriolog- l laboratory,[2] can not readily be carried out at the nic or bedside, making it necessary to prepare the ulsions in advance. There are disadvantages to ueous solutions, whether emulsified or not, regard-

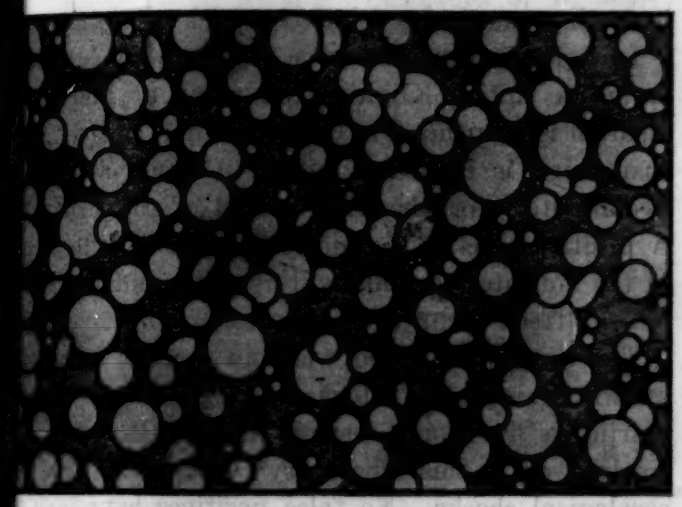

FIG. 1. Schematic drawing of a water-in-oil emulsion aqueous penicillin solution in peanut oil. The peni- lin is contained in the water droplets.

g potency and stability, since they may be affected versely by storage and shipping. The technic to be scribed overcomes these difficulties. In addition it sures the sterility of the preparation, since the pro- dure is carried out in closed, sterile containers.

Water-in-oil emulsions of penicillin can be made as llows: One and four tenths ml of sterile 0.85 per nt. salt solution are drawn into a sterile syringe and ected into a vial containing 100,000 Oxford units dry penicillin. To the penicillin solution 3.1 ml of autoclaved mixture containing 11 parts of a lano- n-like substance[5] and 20 parts peanut oil are added by means of a sterile syringe and needle of 17 gauge. With the needle still inserted through the rubber cap of the vial, the mixture can be readily emulsified by repeated withdrawals and ejections. As soon as the mixture assumes a uniformly creamy, slightly viscid consistency, satisfactory emulsification has occurred and it is ready for injection. Examination of such an emulsion under the microscope shows aqueous droplets of microscopic size in an oily menstruum (Fig. 1).

Water-soluble drugs and biologic products, as well as aqueous suspensions of particulate matter, such as bacteria or viruses, can be emulsified by this technique.

JULES FREUND
K. J. THOMSON

INTRACARDIAC TRANSPLANTATION IN THE URODELE

TRANSPLANTS are sometimes used in determining the extent to which embryonic structures are capable of independent development. Such transplants must not only be provided with adequate nourishment, but they must be effectively isolated from environmental factors which might alter their intrinsic developmental pattern.

In the transplantation sites most commonly used for this purpose the implanted material is exposed to the possible influence of a number of extrinsic factors. The nature and extent of this influence in a given case is not easily determined. Moreover, it is difficult to make a direct comparison between one transplant and others of a similar series because of slight variations in the composition and proximity of the neighboring tissues. Under these conditions the developmental capacity of the implanted tissue is difficult to determine.

It has been found that these experimental conditions can be improved considerably by using the heart cavity as the transplantation site. The method was developed for work with *Ambystoma maculatum,* to which species the following account and comments apply.

The host animals are chosen at a stage when the heart is beating but before the actual circulation of blood has commenced. For this species embryos of Harrison's stage 35 are used. The animals at this stage are not very active. It is therefore unnecessary to use an anesthetic except when it is needed at the same time to quiet an older donor.

The operations are performed under a dissecting microscope after arranging the donor and host in the usual way. The transplantation site is prepared by making a transverse incision through the ventral body

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[3] C. B. Strauch, *Jour. Am. Med. Asn.,* 92: 1177, 1929.
[4] A. Cohn, B. Kornblith, I. Greenstein, K. J. Thomson nd J. Freund. Experiments to be published.
[5] The lanolin-like substance used is sold under the trade me of Falba (Manufactured by Pfaultz and Bauer, Inc., New York, N. Y.); other satisfactory emulsifying agents, producing water-in-oil emulsions, may be used.

wall just anterior to the liver, thus exposing the heart. This incision is then carried through into the sinus venosus. Since the circulation is not yet fully established the resulting hemorrhage soon subsides. The tissue to be implanted, having been previously secured and cached in a depression beside the prospective host, is then inserted into the open heart cavity. The incision closes rapidly and the retention of the implant is assured. The host can then be transferred to the container in which it is to be reared. After an appropriate interval the animal is sacrificed and the graft is recovered by sectioning the heart region.

The development of a number of embryonic tissues has been studied by this method, and it is felt that the encouraging results which have been obtained are in a large measure due to the adaptability of the transplantation site. The heart cavity is easily accessible and can accommodate fairly large pieces of tissue. Operations can therefore be performed rapidly and with little mechanical injury to the donor tissue. Since the incision closes rapidly it is possible to transfer the host without waiting for the implant itself to become fixed. The operation apparently causes no permanent damage to the host, and the mortality is negligible.

The blood plasma undoubtedly serves well to nourish the implant until vascularization has taken place. Thus a favorable environment is provided for the tissue during this critical period.

That a permanent attachment takes place very soon is indicated by the fact that the implants are not carried forward with the onset of circulation but can be recovered very near to their original site. When older hosts are used the implants often are carried as emboli which obstruct the blood supply to one or more gills, but this does not occur when tissues are implanted into the host before the onset of circulation.

The grafts survive in a very high percentage of cases. This indicates that physiologic conditions for the transplant are quickly restored and maintained in the heterotopic site.

Only the tissues of the blood vascular system are included in the environment. These factors are subject to little variation and probably act in a purely nutritive capacity. Thus a near approach to the controlled environment of the culture method is afforded.

ERLING S. HEGRE

DEPARTMENT OF ANATOMY,
MEDICAL COLLEGE OF VIRGINIA

A MODIFICATION OF THE UREASE TEST FOR PROTEUS

THE ability of Proteus species to decompose urea[1] is considered an important identifying characteristic by Bergey,[2] and has recently been recommended by Ferguson *et al.*[3] as a routine differential test in enteric bacteriology.

In the test as employed by the above workers inoculation of suspected organisms into a urea medium is followed by a twenty-four-hour incubation at 37° C. before results are obtained. Since the test is dependent upon the presence of the enzyme urease it seemed apparent that if a large amount of inoculum were employed in a small amount of urea medium decomposition could be quickly determined.

The medium used is that of Ferguson and Hook. It contains 2 per cent. urea (Baker's C.P.), 0.1 per cent. KH_2PO_4, 0.1 per cent. K_2HPO_4, 0.5 per cent. NaCl and 1.0 per cent. C_2H_5OH in distilled H_2O. The pH is adjusted to 7.0. A sufficient quantity of 0.2 per cent. aqueous Phenol Red is added to give a definite color. The medium is sterilized by Seitz filtration. Sterilization is not necessary, however, if the medium is stored in the refrigerator or made up in small amounts for relatively quick utilization.

In order to run the test a heavy inoculum scraped from an initial Kligler Iron slant or other solid medium is suspended in 0.1 ml of urea medium. The suspension is incubated at 37° C. and observed at five-minute intervals for a period of thirty minutes. A positive reaction, as indicated by a definite change of the indicator to pink, will usually occur in the first ten to fifteen minutes.

The test as described was run against a series of known Shigella, Proteus and Salmonella strains. During a five-month period the test has been routinely used in this laboratory[4] with biochemical and serological checks. No false positives have been observed.

THEODORE G. ANDERSON,
Captain, Sanitary Corps,
19th Medical General Laboratory

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